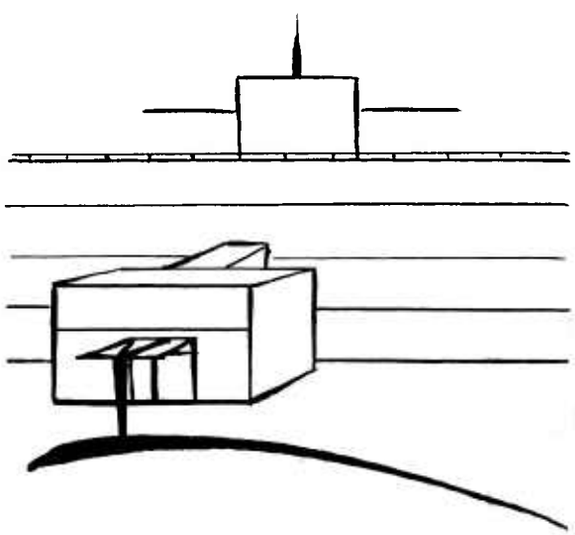
64-10

432215

MEDICAL GENERAL LABORATORY (406)

PROFESSIONAL REPORT

JULY 1962 THROUGH JUNE 1963



United States Army Medical Command, Japan

MEDICAL GENERAL LABORATORY (406)

PROFESSIONAL REPORT

July 1962 through June 1963

United States Army Medical Command, Japan
APO 343, San Francisco, California

INTRODUCTION

This report covers a year marked with change. Funding and personnel reached their lowest level. As the year began, studies were underway to revitalize and redefine the mission of this laboratory. This revitalization was to again result in the formation of the laboratory as a TO&E unit. The mission was to be one of greatly expanded scope in both service and research.

The long history of service and research accomplishments of the 406th Medical Laboratory are well-known throughout the world. Despite personnel and funding limitations this year, many outstanding accomplishments were made because of the esprit de corps of the assigned military and civilian personnel and the continued faithful cooperation of the Japanese people.

JOSEPH F. METZGER
Lt Colonel, MC
Commanding

NOTE

This edition of the Professional Report of the Laboratory covers a reporting period for fiscal year 1963. From 1 July 1962 through 30 June 1963, this Laboratory was still designated the Medical General Laboratory (406). In order to keep our readers informed on the current status of the Laboratory, we wish to announce that on 24 September 1963, General Order Number 123, United States Army Japan, activated the 406th Medical Laboratory as a TO&E unit. The correct mailing address is: Commanding Officer, 406th Medical Laboratory, United States Army Medical Command, Japan, APO 343, San Francisco, California.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

BACTERIOLOGY DEPARTMENT

The Bacteriology Department is the only center of the Armed Forces in the WESTPAC area for:

1. Complete typing of Salmonella, Shigella, Bethesda-Ballerup, Providencia, Klebsiella, and Escherichia coli.

2. Typing of Vibrio.

3. Phage typing of Staphylococcus.

4. Serotyping of Streptococcus.

5. Diagnostic procedures for Leptospira.

6. Classification of Clostridia.

7. Isolation and/or identification of Mycobacteria.

The activities of the Department from July 1962 to June 1963 follow.

Routine Diagnostic Procedures

The Department continued its role as a routine clinical laboratory in support of the U. S. Army Medical Command, Japan, and as a reference laboratory at the medical general laboratory level.

A majority of the routine workload was contributed by the U. S. Army Hospital, Camp Zama, Japan, which relies on this laboratory for all bacteriological studies. The remainder was contributed by other services and authorized installations.

During the period 1 July 1962 through 30 June 1963, a total of 9817 specimens were processed in the Diagnostic Section requiring a total of 1,354,631 performance units. Table 1 shows the source and number of specimens received by the Diagnostic Section for routine culture and/or identification.

There were no significant epidemiological trends noted during the period covered by this report. A short summary of the main pathogens encountered are as follows:

Streptococci, beta hemolytic. Streptococci, beta hemolytic continued to be of high incidence in isolations from throat cultures. From the 6634 specimens received (6292 Army, 8 Air Force, 334 Navy) 1097 yielded Streptococcus, beta hemolytic.

Staphylococci. During the twelve-month period covered by this report a pronounced variation was noted between staphylococcal isolations from urinary tract infections and staphylococcal isolations from other inflammatory sources.

Table 1. The Type and Number of Specimens Received for Routine Diagnostic Bacteriology

Source and/or specimen	No. received
Blood	156
Throat	6,634
Urine	1,100
Pus	303
Body fluid and cavity	45
Urethral	256
Vaginal and cervix	93
Ear	91
Eye	58
Nose	142
Sputum	199
CSF	47
Penile (Darkfield and cultures)	134
Sterility tests	376
Miscellaneous	182
Total	9,816

Of the 1,450 cultures from urinary tract infections, 608 staphylococcus isolates were obtained. Eighteen of these staphylococcus strains were H ≠ S ≠ M ≠ C ≠ (hemolytic ≠, salt ≠, mannitol ≠, coagulase ≠). A majority of the coagulase negative staphylococcus was resistant to penicillin in vitro. Seven thousand seven hundred sixty-one staphylococcus isolates were obtained from other inflammatory sources (throat, ears, nose, pus, body fluid and cavities). The number of H ≠ S ≠ M ≠ C- staphylococcus isolates totaled 728 and the H ≠ S ≠ M ≠ C- staphylococcus isolates totalled 183. From miscellaneous sources, such as fomite tests, conducted by Preventive Medicine, U. S. Army Hospital, Camp Zama, 89 specimens were received by the diagnostic section. Sixty-seven specimens yielded staphylococcus, H ≠ S ≠ M ≠ C-, and two specimens yielded staphylococcus H ≠ S ≠ M ≠ C ≠. Phage typing was performed on the coagulase positive isolates and two of the coagulase negative isolates. The resulting reports were non-typable.

Diplococcus pneumoniae. Six hundred forty-four Diplococcus pneumoniae strains were isolated from specimens submitted for routine bacteriological culture as follows: 595 Throat Cultures, 29 Sputum Cultures, 17 Nose Cultures, 1 Ear Culture and 2 Urine Cultures.

Neisseria gonorrhoeae. Sixty-six N. gonorrhoeae isolates were obtained from 256 specimens submitted for routine bacteriological study from Army, Navy, Marines, Air Force and civilian personnel in the Zama area.

Neisseria intracellularis. In seven cases N. intracellularis was isolated and identified. Three isolates were obtained from cerebrospinal fluid (CSF), three from blood cultures, and one from an ear culture.

Treponema pallidum. One hundred twenty-two patients were referred to this Department for darkfield examination, and in twenty-one of these cases T. pallidum was demonstrated. One very interesting case was observed in a patient who was diagnosed for suspected secondary syphilis and referred to this section for a darkfield examination of a mucoid white patch on the uvula. T. pallidum was demonstrated.

Pleuropneumonia-like-organism (PPLO). PPLO has been found in association with many diseases, both in man and animals; therefore, it is possible that many investigators would accept PPLO as the possible etiological agent when isolated from human sources.

During this report period nineteen PPLO strains were isolated. Twelve strains were isolated from urethral, five from vaginal, and two from penile lesion cultures.

Escherichia coli. Three hundred and forty strains of E. coli from 1,100 urine specimens and 19 strains of E. coli from 94 vaginal specimens were isolated and/or identified by the diagnostic section.

Hemophilus influenzae. Only a relatively few isolates of H. influenzae have been obtained during this report period. Twenty-six isolates were obtained from throat cultures; thirteen from eye specimens; three from nose specimens; one from an ear specimen; one from a pus specimen; one from a CSF specimen; and two from urine cultures.

Klebsiella pneumoniae. This organism was isolated mostly from throat cultures. K. pneumoniae was isolated in a total of 71 cases. Two specimens obtained at autopsy were submitted by the Pathology Department for identification. K. pneumoniae was isolated from both the blood and peritoneal specimens.

ENTERIC SECTION

Routine specimens examined. During the period of July 1962 to June 1963, 507 enteric specimens were received and processed (isolation, identification and/or confirmation). Table 2 shows the number and type of specimens received for bacteriological examination.

Table 2. The Number and Types of Specimens Received

Type of specimen	For isolation	Confirmation and/or identification	Total
Stool	382		382
Rectal swab	76		76
Culture		49	49
Total	458	49	507

Tables 3, 4 and 5 show the respective enteropathogens isolated and identified in the Enteric Section .

Enteric Survey. In addition to the routine workload performed, one enteric survey was conducted on the food handlers at Camp Drake. The purpose of this survey was to locate the carrier of Shigella dysentery. A total of twenty-eight stool specimens were submitted by the Camp Drake Dispensary. Five enteric pathogens were isolated: one Shigella, three pathogenic E. coli, and one Bethesda-Ballerup. Serotypes were as follows:

Isolated organism		Number
Shigella flexneri	2a	1
Bethesda-Ballerup	0-20	1
Escherichia coli	0-75	1
Escherichia coli	0-25	2
Total		5

Salmonella. The Salmonella strains isolated and identified, are listed according to species in Table 3. Five of the six Salmonella strains isolated and identified were strains of Salmonella paratyphi (A). There isolations were made from patients hospitalized in the U. S. Army Hospital, Camp Zama. One strain of Salmonella typhi (Vi) was isolated from a specimen submitted by the 121st Evacuation Hospital, Korea.

Shigella. Twenty Shigella strains were isolated and identified during the period covered by this report. Table 3 shows the number of strains isolated and identified according to species.

Of the sixteen strains isolated, fourteen strains were obtained from specimens submitted by the U. S. Army Hospital, Camp Zama.

One strain of Shigella sonnei 1 and one strain of Shigella flexneri 1a, were isolated from two different normal carriers.

Outbreaks. Nineteen cultures, isolated during an outbreak of dysentery, were received from the Chitose Air Base Dispensary for identification. Most of these cultures were identified as Escherichia coli; no Shigella species were identified.

Five strains of E. coli demonstrated unusual biochemical reactions which were not characteristic of this species. Variations in lactose fermentation and indol formation were noted. The biochemical reaction patterns and the serotypes of these five strains of E. coli are indicated in Table 4.

The five strains of E. coli were agglutinated with Shigella antisera. The results of this test indicated that the patients were probably infected with Shigella; however, since Shigella species were not isolated or identified, a transformation study on E. coli vs. Shigella species was necessary to confirm this observation.

On the basis of the cross reactions observed between the E. coli strains and indicated Shigella antisera, investigation is now underway to determine if Shigella infection can be diagnosed by typing the E. coli, or Proteus morganii, using Shigella typing sera, isolated from specimens of suspected Shigellosis patients.

Table 3. Type and Species Distribution of Shigella and Salmonella Isolated and/or Identified

Strain	Number isolated	Number identified	Total
Shigella flexneri 1a	4	-	4
Shigella flexneri 1b	1	-	1
Shigella flexneri 2a	1	1	2
Shigella flexneri 2b	2	1	3
Shigella flexneri 3b	1	-	1
Shigella flexneri 4a	-	1	1
Shigella sonnei 1	7	1	8
Shigella sonnei 2	-	1	1
Total	16	5	21
Salmonella paratyphi (A)	5	-	5
Salmonella typhi (Vi)	-	1	1
Total	5	1	6

Table 4. Characteristics of Different Strains of Escherichia coli Isolated from Dysentery Patients

Biochemical reactions		Strain 1866	1869	1870	1873	1874
KIA		K/AG	K/AG	K/AG	K/AG	K/AG
H_2S		-	-	-	-	-
Mannitol		(+)	(+)	(+)	(+)	(+)
Motility		+	+	+	+	+
Indol		+	+	+	+	-
Citrate		-	-	-	-	-
V.P.		-	-	-	-	-
M.R.		+	+	+	+	+
Lactose		-	+3	+3	+3	-
Urease		-	-	-	-	-
E. coli serotype		O-25	O-25	O-25	O-25	O-91
Shigella type dysenteriae	1	-	-	-	-	-
	2	-	-	-	-	2+
	3	-	-	-	-	3+
	4	-	-	-	-	4+
	5	-	-	-	-	-
	6	-	-	-	-	-
	7	-	-	-	-	-
flexneri	1	4+	3+	3+	+	4+
	2	1+	+	+	-	1+
	3	2+	2+	2+	2+	4+

Table 4 (Cont'd)

flexneri	4	4+	-	-	-	-
	5	3+	4+	4+	3+	3+
	6	-	-	-	-	3+
	x	4+	4+	3+	2+	2+
	y	2+	1+	±	-	3+
boydii	1	-	-	-	-	2+
	2	-	-	-	-	-
	3	-	-	-	-	2+
	4	-	-	-	-	2+
	5	3+	3+	3+	-	2+
	6	-	-	-	-	-
	7	4+	-	-	-	-
	8	2+	-	-	-	2+
	9	-	-	-	-	-
sonnei	1	-	-	-	-	-
	2	4+	4+	4+	2+	2+

Key: (+), positive with gas; +, positive; -, negative; +3, positive after three days; 1+ .....4+, degree of agglutination; K/AG, alkaline slant and acid butt with gas.

Table 5. Serotype Distribution of Escherichia coli isolated and/or Identified

Serotype		Number Isolated Submitted by Army	Number Identified (Typing Only) Submitted by Army	Navy	Air Force	Total
Pathogenic	0-25	30	4	4	6	44
	0-26	4	-	-	-	4
	0-55	1	-	-	3	4
	0-75	13	2	-	2	17
	0-86	2	-	-	-	2
	0-112	1	-	-	-	1
	0-119	4	1	-	-	5
	0-125	2	-	-	-	2
	0-126	13	-	-	2	15
	0-127	11	-	1	-	12
	0-128	2	-	-	-	2
Total		83	7	5	13	108
Non-Pathogenic		264	8	2	4	278
Total		347	15	7	17	386

Escherichia coli. One hundred eight pathogenic E. coli and 278 non-pathogenic E. coli strains were isolated and identified during the period of July 1962 through June 1963. The number of pathogenic strains; and the number of non-pathogenic strains are listed in Table 5.

Twenty-four strains of E. coli 0-25 and thirteen strains of E. coli 0-75 were isolated from infantile diarrhea specimens.

Two strains of E. coli 0-25 were isolated from dog specimens. These dogs were diagnosed as having enterocolitis by the Veterinary Department of this laboratory. One strain of E. coli serotype 0-127 was isolated from a stool specimen of a premature baby with diarrhea.

Citrobacter (Bethesda-Ballerup). A total of seven Bethesda-Ballerup strains were isolated and identified. Table 6 shows the distribution accuracy to their serotype.

Table 6. The Number of Bethesda-Ballerup Strains Isolated and Identified

Strain	Number Isolated	Number Identified	Total
B-B 0-2	1	-	1
0-1	-	2	2
0-3	1	-	1
0-4	-	2	2
0-20	1	-	1
Total	3	4	7

One strain of Bethesda-Ballerup 0-3 was isolated from a monkey with severe diarrhea in the Bacteriology Department of this laboratory. One strain of Bethesda-Ballerup 0-2 was isolated from an acute gastroenteritis patient. Two strains of Bethesda-Ballerup 0-1 and two strains of Bethesda-Ballerup 0-4 were submitted by the 121st Evacuation Hospital, Korea, for identification of Salmonella.

FOOD AND WATER BACTERIOLOGY

During the period 1 July 1962 to 30 June 1963 a total of 6,070 food and water specimens were processed. Table 7 shows the number of specimens submitted for examination.

Table 7. Number and Type of Specimens Received for Examination

Specimens	Number
Water (tap, well, swimming pool, lake and beach)	2,484
Vegetables	870
Dairy products	1,549
Foods	1,167
Total	6,070

Food Bacteriology. During the period covered by this report, eight food specimens were received for bacteriological examination. These specimens were analyzed for suspected food poisoning. Three staphylococcus isolates were obtained. Further tests revealed positive enterotoxin reaction in animals. (See Table 8).

In routine analysis of food specimens, four staphylococcus strains were isolated. Results obtained in animal tests are shown in Table 9.

Table 8. Results Obtained from Foods Suspected of Causing Food Poisoning

Sample number	Date received	Material	Organisms	Animal results
1	27 Jul 62	Shrimp cocktail (frozen)	No causative organisms	
2	30 Jan 63	Meat from stew	Staph. H+ S+ M+ C+	Enterotoxin reaction positive in cats
3	2 Apr 63	Beefsteak	Staph. H+ S+ M+ C-	Negative animal reaction
4	16 Apr 63	Ham, smoked	Staph. H+ S+ M+ C+	Enterotoxin reaction positive in cats
5	2 May 63	Ham, smoked	No causative organisms	
6	9 May 63	Meat, fresh	Staph. H+ S+ M+ C+	Kitten inoculation; negative results
7	4 Apr 63	Chili, canned	No food poisoning organisms isolated	
8	31 May 63	Seafood cocktail, bottled	Bacillus cereus	Weakness and nausea 30 minutes after feeding to kittens

Table 9. Organisms Isolated from Routine Samples [1,2]

Sample number	Date received	Material	Organisms	Animal results
9	6 May 63	Inflight meal, frozen	Staph. H/ S/ M/ C/	Kitten inoculation; negative results
10	13 May 63	Chocolate syrup, canned	Clostridium perfringens	Mouse inoculation; negative results
11	9 May 63	Chocolate syrup, canned	Clostridium perfringens	Mouse inoculation; negative results
12	17 Jun 63	Lobster cocktail, bottled	Clostridium perfringens	Mouse inoculation; negative results

[1]References: Hammon, William Mcd., M.D., Ph.D., Staphylococcus enterotoxin: An improved cat test, chemical and immunological studies. Am. J. of Pub. Health, Vol 31, Nov 1941.

[2]Dack, G. M., H. Sugiyama, Owens, Francis J., Kirsner, Joseph B. Failure to produce illness in human voltuneers fed Bacillus cereus and Clostridium perfringens. The Journal of Infectious Disease , Vol 94, 34, 1954.

The number of food spoilage organisms isolated and identified from various foods is shown in Table 10.

Dairy Products Bacteriology. Table 11 gives the results obtained from bacteriological examination of milk, ice cream, and cottage cheese.

Vegetable Bacteriology. A total of 867 vegetable samples were examined for the presence of Escherichia coli. Table 12 shows the monthly variation of E. coli findings in vegetables.

Water Bacteriology. During the period July 1962 to June 1963 a total of 2,534 water specimens were examined by membrane filter technique. The correlation tables were compiled to compare the standard dilution tube method of 1961 and the membrane filter technique of 1962 and 1963 for coliform bacteria findings in drinking water specimens. Table 13 shows correlation of coliform findings in drinking water samples.

Table 10. The Number of Food Spoilage Organisms Isolated from Various Foods [1,2]

Organism	Frozen foods	Meat, fresh (hamburger)	Sea foods (fresh frozen)	Ham, smoked	Sea food products	Meat products (canned)	Fish products (canned)	Tomato products (canned)	Fruits, frozen (canned)	Fruit juice (bottled)	Dog food	Others	Totals
Staphylococcus H+,S+,M+,C-	2	2	1	2									7
H+,S+,M+,C+	1			1							1		3
Streptococcus alpha-hemolytic		3		2							1	4	10
Pseudomonas aeruginosa	3		1	1									5
E. coli(055)											1		1
E. coli(025)	1												1
E. coli(non-Pathogen)	4	3											7
Proteus morganii		1											1
Proteus rettgeri		2											2
Proteus vulgaris		1											1
Coliform bacteria	7	6										1	14
Paracolobactrum	1	7	1								1	1	11
Cl. sporogenes						2							2
Bacillus sp.					1	1	2		1	2		1	8
Lactobacillus sp.					5								5
Leuconostoc sp.					2			2					4
Saccharomyces sp.		1			1				2	13			17
Torula sp.										1			1
Candida sp.									2	2			4
Penicillium sp.		1								4			5
Aspergillus sp.									1	2			3
Byssochlamys sp.									1	1			2
Mucor sp.										2			2

[1]References: Hammon, William Mcd., M.D., Ph.D., Staphylococcus enterotoxin: An improved cat test, chemical and immunological studies. American Journal of Public Health, Vol 31, Nov 1941.

[2]Dack, G.M., H. Sugiyama, Francis J. Owens, Joseph B. Kirsner. Failure to produce illness in human voltuneers fed Bacillus cereus and Clostridium perfringens. The Journal of Infectious Diseases, Vol 94, 34, 1954.

Table 11. Monthly Variation of Coliform Findings and High Plate Count in Dairy Products

Milk and ice cream

Month 1962	Number of samples	Number of coliform positive	Per cent of total	Number of SPC over 50,000/ml.	Per cent of total
Jul	116	14	12.1	4	3.4
Aug	112	3	2.6	1	0.9
Sep	95	4	4.2	2	2.1
Oct	102	2	2.0	1	1.0
Nov	104	2	1.9	1	1.0
Dec	78	1	1.3	1	1.3
1963					
Jan	123	1	0.8	0	-
Feb	103	1	1.0	1	1.0
Mar	155	0	-	0	-
Apr	170	4	2.4	3	1.8
May	113	3	2.7	0	-
Jun	113	7	6.2	0	-
Total	1,384	42	3.04	14	1.08

Cottage Cheese

Month 1962	Number of samples	Number of Coliform over 50/gram.	Per cent of total	Number of Mold and Yeast over 50/gram.	Per cent of total
Jul	17	4	23.5	7	41.2
Aug	11	2	18.2	0	-
Sep	6	1	1.6	0	-
Oct	7	0	-	0	-
Nov	10	0	-	0	-
Dec	6	0	-	0	-
1963					
Jan	11	0	-	0	-
Feb	20	3	15.0	0	-
Mar	20	0	-	0	-
Apr	23	1	4.3	2	8.7
May	13	2	15.4	1	7.7
Jun	16	2	12.5	1	6.3
Total	160	15	9.37	11	6.87

Table 12. Monthly Variation of E. coli Findings in Vegetables

Samples	1962 Jul	Aug	Sep	Oct	Nov	Dec	1963 Jan	Feb	Mar	Apr	May	Jun	Per cent of totals	Totals
Lettuce														
No. of Samples	19	17	11	10	3	14	12	19	9	25	11	1		151
No. of E. coli	5	1	1	1	0	1	0	0	1	1	0	0	7.2	11
Parsley														
No. of Samples	6	6	10	2	3	2	2	8	8	14	7	0		68
No. of E. coli	1	0	4	0	1	0	0	0	0	0	0	0	8.8	6
Cabbage														
No. of Samples	19	13	14	6	7	9	12	19	9	16	10	1		135
No. of E. coli	1	0	0	0	0	0	0	0	0	0	2	1	3.0	4
Onions														
No. of Samples	13	15	10	9	5	11	11	21	8	22	14	1		140
No. of E. coli	0	0	2	0	0	0	0	0	0	0	1	0	2.1	3
Radishes														
No. of Samples	4	2	6	4	2	5	4	9	2	13	2	0		53
No. of E. coli	2	1	0	1	1	0	0	0	0	1	1	-	13.2	7
Celery														
No. of Samples	8	8	7	4	3	9	6	15	6	13	1	1		81
No. of E. coli	1	0	0	0	0	0	0	0	0	0	0	0	1.2	1
Cucumbers														
No. of Samples	4	2	4	2	1	6	3	3	0	4	6	0		35
No. of E. coli	0	1	0	0	0	0	0	0	0	0	0	0	2.9	1
Carrots														
No. of Samples	2	2	1	3	2	7	4	11	1	13	7	0		53
No. of E. coli	0	0	0	0	0	0	0	0	0	1	0	-	1.9	1
Tomatoes														
No. of Samples	6	6	11	8	3	8	5	13	1	13	5	0		79
No. of E. coli	0	0	0	0	0	0	0	1	0	0	0	-	1.3	1
Others														
No. of Samples	9	5	16	10	3	2	1	3	11	5	7	0	5.6	72
No. of E. coli	2	0	1	1	0	0	0	0	0	0	0	-		4
Total Samples	90	76	90	58	32	73	70	121	55	138	70	4		867
Total E. coli	12	3	8	3	2	1	0	1	1	3	4	1		39

Table 13. The Correlation Table of Standard Dilution Tube Method (1961) and Membrane Filter Technique (1962, 1963) of Coliform Findings in Drinking Walter Specimens

Months	No. of Samples			No. of Coliform Pos.			Per cent of Total		
	1961	1962	1963	1961	1962	1963	1961	1962	1963
Jan	334	385	214	4	3	4	1.2	0.8	1.9
Feb	439	388	184	1	1	3	0.2	0.3	1.6
Mar	333	312	187	1	0	0	0.3	-	-
Apr	303	323	168	2	3	1	0.7	0.9	0.6
May	389	279	137	2	0	2	0.5	-	1.5
Jun	330	267	190	5	5	2	1.3	1.9	1.1
Jul	397	315		5	2		1.3	0.6	
Aug	372	221		1	3		0.3	1.4	
Sep	369	220		3	7		0.8	3.2	
Oct	307	162		1	5		0.3	3.1	
Nov	417	175		2	2		0.5	1.1	
Dec	340	164		1	0		0.3	-	
Totals	4,330	3,211	1,080	28	31	12	0.64	0.97	1.10

TUBERCULOSIS SPECIMENS

The Department continued its operation as a tri-service reference laboratory for Mycobacteria. Table 14 shows the number and types of specimens received for Mycobacterium tuberculosis examination. The results obtained from examination for Mycobacterium tuberculosis is given in Table 15. (Thirty-eight bioassay specimens are not included in Table 15).

A total of 1,566 specimens were submitted for bacteriologic diagnosis of Mycobacterium tuberculosis by smear and culture. Of these 228 specimens (14.56 per cent) were positive for Mycobacterium tuberculosis and 1,338 specimens (85.44 per cent) were negative.

Concentrations of 885 sputum specimens were examined microscopically and by culture. A discrepancy in findings is sometimes observed when smear and culture examinations are compared. Table 16 shows the difference in microscopic and culture findings.

Table 17 shows the distribution of isoniazid (INH) concentration in sera of Japanese female patients receiving INH and Para-Aminosalicylic Acid (PAS).

Table 14. Number and Types of Specimens Received and Processed for *Mycobacterium tuberculosis*

Type of specimen	Army	Air Force	Navy	Total
Sputums	875	3	7	885
Gastric contents	250	-	1	251
Body fluids	102	-	-	102
Tissues	29	-	-	29
Urines	239	-	5	244
Pus	31	-	-	31
Culture for identification	22	2	-	24
Isoniazid-serum bioassay	-	-	38	38
Total	1,548	5	51	1,604

Table 15. Results of Examination for *Mycobacterium tuberculosis* Performed on Various Types of Specimens

	Results of examination for M. tuberculosis					
	Positive		Negative		Total	
Type of specimen	No.	Per cent	No.	Per cent	No.	Per cent
Sputum	149	16.84	736	83.16	885	100
Gastric contents	25	9.96	226	90.04	251	100
Body fluid	7	6.86	95	93.14	102	100
Tissue	3	10.34	26	89.66	29	100
Urine	15	6.14	229	93.86	244	100
Pus	8	25.80	23	74.20	31	100
Culture for identification	21	87.50	3	12.50	24	100
Total	228	14.56	1,338	85.44	1,566	100

Table 16. Comparison of Microscopic and Culture Findings on Sputum Specimens Submitted for Isolation of *Mycobacterium tuberculosis*

	Culture					
	Positive		Negative		Total	
Microscopic	No.	Per cent	No.	Per cent	No.	Per cent
Positive	123	74.54	42*	25.46	165	100
Negative	26*	3.61	694	96.39	720	100
Total	149	16.84	736	83.16	885	100

*Twenty-six of the 149 sputum specimens yielding positive cultures for *Mycobacterium tuberculosis* had negative concentrated smears. In all twenty-six examinations where growth showed less than five colonies, the discrepancy can be attributed to the very small number of tubercle bacilli in the inocula of the

specimens. Of the forty-two smear-positive, culture-negative specimens, ten were contaminated or incurred unknown partial changes of media during incubation. These ten specimens were obtained from patients who previously had positive smears and cultures. Nineteen of the smear-positive, culture-negative sputum specimens contained only rare acid-fast bacilli. It can only be assumed that the acid-fast bacilli were probably drug-sterilized.

Table 17. Distribution of Serum Isoniazid Concentrations in Female Japanese Tuberculosis Patients Receiving INH* and PAS**

Serum isoniazid concentration mcg INH/ml serum	Number of hours after receiving drug: 2 hours — No. of patients	2 hours — Per cent	6 hours — No. of patients	6 hours — Per cent
1.80	12	60.00	-	-
0.90	8	40.00	10	55.56
0.45	-	-	6	33.33
0.23	-	-	2	11.11
0.15	-	-	-	-
Total	20	100.00	18	100.00

*INH is given in dosage of 10 mgms/kg/day
**PAS is given in dosage of 12 gms/day

MYCOLOGICAL ACTIVITIES

A total of 330 specimens were received for isolation and/or identification. Table 18 shows the sources and number of specimens received from the contributing services.

Table 19 shows the number and identification of mycological specimens received.

Table 18. The Number and Type of Specimens Received for Mycological Examination

Source	Army	Air Force	Navy	Other	Total
Skin	66	0	3	0	69
Nail	2	0	1	0	3
Hair	7	0	0	0	7
Vaginal	99	0	3	0	102
Penis	7	0	0	0	7
Urethral	5	0	0	0	5
Sputum	23	1	2	1	27
Urine	18	0	0	0	18
Stool	6	0	0	0	6
Pus	1	0	1	0	2
Spinal fluid	14	0	0	0	14
Ear	5	0	1	0	6
Gastric	7	3	0	0	10
Other	21	1	32	0	54
Total	281	5	43	1	330

Table 19. The Number and Identification of Mycological Specimens Received

Genus and speces	Army	Air Force	Navy	Others	Totals
Candida albicans	69	0	1	1	71
Trichophyton rubrum	0	1	0	0	1 2
Epidermophyton floccosum	2	0	0	0	
Trichophyton mentagrophytes	0	0	0	1	1
Microsporum furfur	4	0	0	0	4
Nocardia brasiliensis	0	2	0	0	2
Total pathogenic fungi	75	3	1	2	81
Number of non-pathogenic fungi	54	1	13	0	68
Number showing negative growth	152	1	28	0	181
Total	281	5	42	2	330

LEPTOSPIRAL ACTIVITIES

Until March 1962, twenty-three species of Leptospiral were used as antigens in the diagnostic agglutination-lysis test. However, Doctor A. J. Alexander, Walter Reed Institute of Research, Washington, D. C., recommended that, for this particular geographical area, the number of antigens be reduced to sixteen. Of the sixteen Leptospiral antigens currently used, twelve are strains originally used while four are additions (L. wolffi, L. celledoni, L. butembo, and L. borincana). Listed below are Leptospiral antigens currently used for the agglutination-lysis test:

L. icterohaemorrhagiae
L. canicola
L. bataviae
L. pomona
L. australis ballico
L. autumnalis Akiyami A
L. ballum
L. alexi
L. hebdomadis
L. hyos
L. grippotyphosa Moscow V
L. wolffi
L. djasiman
L. celledoni
L. butembo
L. borincana

Table 20 summarizes the sources of specimens received from contributing services for Leptospiral agglutination-lysis tests.

Table 21 shows positive and negative results obtained from submitting services and sources of serum tested for agglutination-lysis procedure.

Table 22 shows number and source of sera which gave positive agglutination-lysis titer against known Leptospiral antigens.

Table 23 shows the number of specimens received from the three different services and authorized agencies for febrile agglutination titrations. The results of the febrile agglutination titrations performed on sera submitted by the four groups are summarized in Tables 24, 25, 26 and 27. A majority of the specimens were received without the necessary clinical information; therefore, no interpretation can be made from the results of the tests.

Table 20. Serum Specimens Received for Leptospiral Agglutination-lysis Test. Positive and Negative Results as Indicated Against One or More of 16 Test Antigens.

Sources of serum	Number of specimens received			
	Army	Air Force	Navy	Total
Patients	87	65	28	180
Animals	3	1	1	5
Total	90	66	29	185

Table 21. Results of Leptospiral Agglutination-lysis Tests

Sources of serum	(Positive)				(Negative)			
	Army	Air Force	Navy	Total	Army	Air Force	Navy	Total
Patients	1	1	1	3	86	64	27	177
Animals	0	0	0	0	3	1	1	5
Total	1	1	1	3	89	65	28	182

Table 22. Number and Source of Sera Which Gave Positive Agglutination-lysis Titer Against Known Leptospiral Antigens.

Services	Leptospiral antigens	Titer	Sources of serum
Army	L. canicola	1:800	Human sera
Air Force	L. ballum	1: 50	Human sera
Navy	L. grippotyphosa Moscow V	1: 50	Human sera

Table 23. Serum Specimens Received for Febrile Agglutination

Number of specimens received				
Army	Air Force	Navy	Others	Total
96	10	21	1	128

Table 24. Number and Titers of Specimens Received for Febrile Agglutination (Army)

Antigens	Negative	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
E. typhosa(H)	8	1	12	20	27	9	6	1	1
E. typhosa (O)	17	17	21	16	13	1	0	0	0
S. paratyphi	16	6	18	28	11	4	2	0	0
S. schottmuelleri	11	8	14	19	18	11	2	2	0
Proteus OX-19	91	2	0	0	0	0	0	0	0
Proteus OX-2	75	11	2	0	0	0	0	0	0
Proteus OX-K	45	31	16	2	0	0	0	0	0
Br. abortus	59	15	5	6	1	0	0	0	0
P. tularensis	91	0	0	0	0	0	0	0	0

Table 25. Results of the Febrile Agglutination Titrations Performed on Sera Submitted by Air Forces

Antigens	Negative	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
E. typhosa (H)	0	0	1	4	1	3	1	0	0
E. typhosa(O)	0	4	2	1	2	0	1	0	0
S. paratyphi	0	3	1	2	3	1	0	0	0
S. schottmuelleri	1	1	0	4	2	2	0	0	0
Proteus OX-19	10	0	0	0	0	0	0	0	0
Proteus OX-2	6	3	1	0	0	0	0	0	0
Proteus OX-K	0	4	4	2	0	0	0	0	0
Br. abortus	4	4	1	1	0	0	0	0	0
P. tularensis	10	0	0	0	0	0	0	0	0

Table 26. Results of the Febrile Agglutination Titrations Performed on Sera Submitted by Navy

Antigens	Negative	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
E. typhosa(H)	1	1	2	4	9	3	1	0	0
E. typhosa(O)	3	5	5	3	4	0	0	1	0
S. paratyphi	6	1	5	6	1	1	1	0	0
S. schottmuelleri	1	1	4	4	6	3	1	1	0
Proteus OX-19	19	2	0	0	0	0	0	0	0
Proteus OX-2	17	4	0	0	0	0	0	0	0
Proteus OX- K	8	5	7	1	0	0	0	0	0
Br. abortus	17	2	1	1	0	0	0	0	0
P. tularensis	21	0	0	0	0	0	0	0	0

Table 27. Results of the Febrile Agglutination Titrations Performed on Sera from Other Sources

Antigens	Negative	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
E. typhosa (H)	0	0	0	1	0	0	0	0	0
E. typhosa (O)	0	0	1	0	0	0	0	0	0
S. paratyphi	0	0	0	0	1	0	0	0	0
S. schottmuelleri	0	0	0	0	0	0	1	0	0
Proteus OX-19	1	0	0	0	0	0	0	0	0
Proteus OX-2	0	1	0	0	0	0	0	0	0
Proteus OX-K	0	1	0	0	0	0	0	0	0
Br. abortus	1	0	0	0	0	0	0	0	0
P. tularensis	1	0	0	0	0	0	0	0	0

OTHER ACTIVITIES

Antistreptolysin "O" Titration. The number and titer of the serum specimens received for antistreptolysin "O" titrating are shown in Table 28. A majority of the specimens were received without the necessary clinical data; therefore, no interpretation can be made from the results of the tests.

Table 28. Number and Titer of Serum Specimens Received for Anti-streptolysin-O-titration.

Services	Titer 1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:1280	Total
Army	2	3	2	18	27	17	15	4	1	1	90
Air Force	0	0	0	0	3	1	2	0	0	0	6
Navy	0	1	4	20	35	35	10	3	0	1	109
Total	2	4	6	38	65	53	27	7	1	2	205

ANIMAL SECTION

The animal section functions as a supply point for animals and rabbit blood requisitioned by various departments in the Medical General Laboratory (406) and other supported installations. Table 29 shows the number of animals supplied and Table 30 shows animal blood issued by this department.

Table 29. Number of Animals Supplied

Month	Other Departments Rabbit	G.P	Hamster	Mice	Bacteriology Department Rabbit	G.P.	Hamster	Mice
July	23	6	-	130	4	10	-	-
August	43	2	4	50	14	3	6	100
September	28	3	-	-	6	-	-	-
October	26	-	-	-	14	2	-	30
November	41	7	-	-	6	2	16	200
December	19	-	-	-	5	3	8	10
January	37	-	-	-	15	-	-	150
February	55	12	-	-	3	2	-	123
March	29	5	-	-	10	13	6	53
April	28	5	-	-	16	8	3	15
May	34	4	-	-	5	-	-	20
June	41	-	-	-	-	3	3	65
Total	404	44	4	180	98	46	42	766

Table 30. Rabbit Blood Issued

Month	Rabbit Blood Issued
July	824.0 cc
August	968.0 cc
September	552.0 cc
October	252.0 cc
November	27.0 cc
December	160.0 cc
January	875.0 cc
February	35.0 cc
March	30.0 cc
April	40.0 cc
May	615.0 cc
June	25.0 cc
Total	4,403.0 cc

At the present time the Bacteriology Department has the following animals:

Rabbit	112	Hamster	52
Guinea pig	50	Cat	2
Mice	226	Monkey	2

Table 31 shows the monthly animal population.

Table 31. Monthly Animal Population

Month	Rabbit	G.P.	Mice	Hamster	Monkey	Cat
July	151	32	272	37	3	1
August	148	31	181	44	3	1
September	142	30	200	50	1	4
October	128	32	228	45	1	1
November	129	33	245	38	1	1
December	130	43	261	32	1	1
January	139	52	236	41	1	1
February	134	50	236	44	3	1
March	131	51	215	67	5	1
April	137	43	247	67	4	1
May	131	35	213	42	2	2
June	120	43	197	42	2	4

RESEARCH AND DEVELOPMENT

The following reports cover activities performed with the research and development funds supplied by the U. S. Army Medical Research and Development Command, Office of The Surgeon General.

Electron Microscopy of *Neisseria gonorrhoeae* During Penicillin Exposure

There has been some controversy as to the emergence of penicillin-resistant *Neisseria gonorrhoeae* in the past few years.

A number of reports indicated that the gonococcus was becoming increasingly resistant to the action of penicillin. Thayer *et al.* (1957) found the minimal inhibitory concentration of penicillin for *N. gonorrhoeae* to be 1.5 to 10 times greater than the previously reported value. Craddock-Watson *et al.* (1958) and Curtis *et al.* (1958) reported a twenty to thirty-fold increase in the resistance of the gonococcus to penicillin. These findings were complemented by clinical reports of penicillin failures in cases of gonorrhea. Mead *et al.* (1960) reported eleven cases which were clinically resistant to penicillin therapy. Six of the eleven cases were reported to have a gonococcus which demonstrated *in vitro* resistance to penicillin by the disc method.

Contrary to this, studies by Sanders *et al.* (1961, 1962) indicated that in some cases of gonorrhea: (a) a synergistic relationship existed between staphylococci and gonococci, thereby producing a penicillin-resistant urethritis; (b) an active substance produced by the staphylococcus is penicillinase-like in action; and (c) that none of the cases which failed to respond to treatment were infected with gonococci that were resistant to penicillin *in vitro* by the disc method.

Another concept proposed for the mechanism of bacterial resistance of antibiotics is the transformation to the L-form. Dienes (1940) and Brown et al. (1942) have reported L-forms of N. gonorrhoeae. An L-form of the gonococcus was induced when strain 193 was exposed to various penicillin concentrations ranging from 4 to 256 units/ml by Barile et al. (1959). None of these investigators have reported the revision of the gonococcal L-form to the vegetative cell upon the removal of penicillin. This would be necessary for completion of the cycle if N. gonorrhoeae employs the resistance mechanism.

For these reasons it was decided to study the effect of penicillin of individual gonococci by electron microscopy.

MATERIALS AND METHODS

N. gonorrhoeae, strain 38124, was used for the study. The organisms were cultured on blood agar plates for twenty-four hours to procure sufficient colonies, which were transferred to brain-heart infusion (BHI) broth containing varying concentrations of procaine penicillin G. At approximately eighteen-hour intervals, cultures for vegetative gonococci and gonococcal L-forms were made on pleuropneumonia-like-organisms (PPLO) plates, blood agar plates, and in BHI broth and BHI broth with a 3 per cent NaCl addition. Isolation of either viable vegetative cells or L-forms was readily accomplished on one or more of these media. The remaining cells were then harvested by centrifugation, fixed with osmium tetroxide, and stained with uranyl acetate. After dehydration in graded concentrations of ethanol, the cells were embedded in a mixture of 80 per cent N-butyl methacrylate and 20 per cent methyl methacrylate. Sections were cut on a Servall "Porter-Blum" ultramicrotome. The sections were viewed and electron micrographs were taken with a JEM-6C electron microscope.

RESULTS

The control series were quite similar in appearance throughout the sixty-five-hour period. A thread-like structure in the center and the granular structure near the cytoplasmic membrane showed little variation.

All the gonococci, which were treated with 10 units of penicillin/ml or a greater concentration, varied morphologically within seventeen hours. A coagulating of nuclear material was apparent. The formation of small intracellular bodies was observed. Some of the cells appeared similar to the "giant cells" observed in other penicillin treated Gram negative bacteria. All cultures were negative for vegetative gonococci after a thirty-minute exposure to 10 units or a greater concentration of penicillin. The emergence of L-forms of the gonococcus occurred after seventeen hours' exposure. Typical L colonies were apparent and stained agar blocks revealed numerous large bodies and granular elements. Subcultures were maintained with little difficulty. The L-forms were resistant to 1000 units of penicillin/ml. A revision of these L-forms to vegetative gonococci was accomplished by the addition of 3.0 per cent Bacto yeast extract and the graded reduction of the NaCl concentration.

Comparative Studies of Laboratory Methods in the Isolation of Mycobacterium tuberculosis

by

T. S. Rei, K. Kimoto, K. Matsuda and P. K. McIlwain

Numerous comparative studies and reviews of the efficiency of various artificial media, as well as animal inoculation, in the isolation of Mycobacterium tuberculosis have been conducted. [3, 5] One of the main problems encountered has been growth promotion on artificial media when only small numbers of bacilli are present in the inocula. The development of fastidious strains of Mycobacteria due to the intensive use of chemotherapy in the treatment of tuberculosis has further added to the problem of isolation. This survey is concerned with the comparison of some of the currently used media in the isolation of extremely small numbers of tubercle bacilli and the routine isolation of M. tuberculosis during chemotherapy.

MATERIALS AND METHODS:

Mycobacterium tuberculosis, strain H37Rv was used for the first phase of the study. Finely dispersed suspensions growing in 7H9 Tween albumin liquid media, were prepared according to the method of Fenner et al. [2] Serial dilutions of this inoculum were made to final concentrations of $5x10^{-5}$, $1x10^{-5}$, $5x10^{-6}$, and $1x10^{-6}$. Five individual drops of 0.02 ml were placed on each plate with 0.2 ml pipettes. Ten plates of each media were used for each dilution. The media employed were as follows:

1) Oleic acid - albumin agar (7H900A Middlebrook)[4].
2) Blood glycerin penicillin agar (Tarshis BAP)[7].
3) Lowenstein-Jensen Egg media (L-J Egg)
4) American Trudeau Society media (ATS)
5) Croft's Modified Hohn media (Mod. Hohn)

After 3 weeks incubation at 36°C, the colonies were counted at a magnification of X25. Each total plate count was considered as one determination. The mean colony count was derived from the 10 plates used for each dilution.

The second phase of the study involved a comparison of 7H900A and Tarshis BAP media in the isolation of tubercle bacilli from positive specimens of patients receiving anti-tuberculosis drugs. Concentrates of both sputum and gastric washings were used as inocula. Three tubes of each media were inoculated with 0.5 ml of each sediment.

The third phase of the study was the comparison of artificial media and guinea pig inoculation in the isolation of M. tuberculosis under routine diagnostic conditions. A total of 603 positive specimens were utilized. Combinations of Lowenstein-Jensen's egg media, Tarshis BAP media, and Petragnani's media were used. Two tubes of each culture media and 2 guinea pigs were inoculated from each specimen. The cultures were incubated and observed weekly for a period of 8 weeks, and the guinea pigs autopsied 8 weeks post injection. Organs displaying gross lesions were removed and the acid-fast bacilli observed microscopically. If the tubercle

bacillus was isolated by culture and the guinea pig inoculation was negative, the pathogenicity of the strain was rechecked in another guinea pig. In such cases, if the second guinea pig inoculation was positive the results were still recorded as culture positive, GP-negative, because of the initial findings.

RESULTS:

The plate counts obtained from the successive dilutions of the H37Rv strain of M. tuberculosis are shown in Table 1. The 7H900A media appeared the most sensitive to the small inocula employed. Tarshis BAP media was somewhat less sensitive than the 7H900A media, but yielded a greater number of viable bacterial units than did the 3 egg media.

The 7H900A media was decidedly superior to Tarshis BAP media in the isolation of tubercle bacilli during Chemotherapy (Table 2). It was noted that the success rate of the 7H9 media was much greater than the BAP from inocula having small numbers of bacilli. The time required for gross detection of the colonies was similar for the 2 media. Approximately one half of the cultures could be read in 15-21 days and the remainder by the fifth week.

The results of the 3rd phase of the trial are shown in Table 3. Complete correlation between cultural techniques and animal inoculation, both being positive, occurred approximately 80 per cent of the time. In the series where only conventional egg media were employed, guinea pig inoculation was somewhat superior, whereas in the series where blood agar penicillin and egg media were utilized, the cultivation technique proved more sensitive. If animal inoculation had not been utilized 8.7 per cent of the positive findings would not have been made. Cultural techniques accounted for the isolation of 11.6 per cent of the strains which gave negative results by guinea pig inoculation.

DISCUSSION:

This survey is in agreement with the findings of others. [4, 6, 8.] Conventional egg media is not as sensitive as BAP or 7H900A in the growth promotion of small numbers of M. tuberculosis. This growth failure is perhaps due, not to lack of any nutritional elements, but rather to the presence of inhibitory substances such as lysozyme. The BAP media was not suitable in the isolation of the tubercle bacillus during chemotherapy. The agents used were combinations of P-aminosalicylate, Isoniazid, and Streptomycin. The greatest percentage of failures occurred when few bacilli were present in the inoculum. The 7H900A media was much more sensitive although growth did not occur from 22 of the 325 specimens. The cultural technique was somewhat more sensitive than guinea pig inoculation in isolating tubercle bacilli when using combinations of commonly employed media. Animals are undoubtedly useful in the conformation of a clinical diagnosis of tuberculosis, however, it has been shown that lesions of the internal organs are not produced by less than a minimal number of 10 organisms. [1.]

Table 1. Comparative Studies on the Efficiency of Artificial Media in the Isolation of Small Numbers of M. tuberculosis

Bacterial Units per Milliliter	7H900A [1]		Tarshis BAP [2]		L-J Egg [3]		A.T.S. [4]		Mod. Hon's [5]	
	Mean	*S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
$5x10^{-5}$	> 200	-	123	4.69	112	5.00	100	5.09	103	5.47
$1x10^{-5}$	73	3.56	30	2.72	23	3.30	20	3.53	19	3.37
$5x10^{-6}$	44	2.10	15	2.00	12.5	2.10	12	2.70	12	2.64
$1x10^{-6}$	8	0.64	5.8	0.74	5.1	1.02	<2	-	<2	-

*Standard Deviation

Table 2. Comparative Studies of 7H9 Oleic Acid Albumin Agar and Tarshis Blood Agar Penicillin Media in the Isolation of M. tuberculosis during Chemotherapy

Media	*Total No. of specimens	Total Number of Colonies per 3 tubes of Media						Total No. of isolations
		1-5	6-10	11-20	21-30	31-50	> 50	
7H900A	325	77	29	18	25	81	73	303
Tarshis BAP	325	45	23	15	14	66	69	232

*Of the 325 specimens, 127 were gastric washings.
Of 198 concentrated sputum smears, 121 displayed Acid-fast bacilli, and 77 were negative.

Table 3. Comparative Studies of Cultural Methods and Guinea Pig Inoculation in the Isolation of M. tuberculosis from 603 Positive Specimens

Media	Guinea Pig Inoculation		Cultivation		Both Methods Positive	Total Number of Specimens
	Positive	Failed	Positive	Failed		
Petragnani and Lowenstein-Jensen	228 (94.2 per cent)	14 (5.8 per cent)	205 (84.8 Per cent)	37 (15.2 per cent)	191 (78.9 per cent)	242
Tarshis BAP and Petragnani	110 (85.3 per cent)	19 (14.7 per cent)	123 (95.4 per cent)	6 (4.6 per cent)	104 (80.6 per cent)	129
Tarshis BAP and Lowenstein-Jensen	199 (85.8 per cent)	33 (14.2 per cent)	217 (93.6 per cent	15 (6.4 per cent)	184 (79.3 per cent)	232
Total	537 (88.4 per cent)	66 (11.6 per cent)	545 (91.3 per cent)	58 (8.7 per cent)	479 (79.6 per cent)	603

The emergence of tubercle bacilli with altered nutritional requirements and animal pathogenicity does pose an important diagnostic problem. Although recently developed cultural techniques offer considerable promise in the cultivation of M. tuberculosis, the use of combinations of media as well as animal inoculation still appear necessary for the accurate laboratory diagnosis of tuberculosis.

BIBLIOGRAPHY:

1. Barnett, M., Bushley, S. R. M. and Mitchison, D. A., Lancet 1, 314 (1953).

2. Fenner, F., Martin, S. P. and Pierce, C. H., Ann. N.Y. Acad. Sci. 52(5), 751 (1949).

3. Middlebrook, G. and Cohn M. L., Am. J. Public Heath 48, 844 (1958).

4. Middlebrook, G., Cohn, M.L. and Schaeffer, W. B., Am. Rev. Tuberc. 70, 852 (1954).

5. Reed, R. W. and Morgante O., Am. J. Med. Sci. 231, 320 (1956).

6. Roberts, E. G., Wallace, J. L., and Ehrlich, H., Am. Rev. Tuberc. 61, 4, (1950).

7. Tarshis, M. S., Weed, L. A., Kinsella, P. C., Parker, M. V. and Dunham, W. B., Am. J. Pub. Health 45, 1157 (1955).

8. Whitcome, F. C., McRoberts, C. C., Norman, J. O. and Halpert, B., Am. Rev. Tuberc. 71, 762, (1955).

The Possible Role of iota Toxin of Type E Clostridium perfringens (L Forms) in the Production of Symptomatology Similar to Hemorrhagic Fever

by
Toshio Kawatomari

Description:

The purpose of this phase of the study is to develop a method for the separation of iota toxin from the culture broth of type E Clostridium perfringens (bacillary form). The partially purified toxin was chromatographed on a DEAE cellulose column to separate the various antigenic components.

Progress:

A preparation of partially purified iota toxin (Method is described in the 1962 Annual Professional Report, MGL (406)) was administered to rabbits intravenously in order to produce anti-iota sera. When the anti-iota serum was tested against the partially purified material by the agar-gel diffusion method, 4 precipitin bands developed. The partially purified toxin was then chromatographed on a column of DEAE cellulose to separate the individual antigenic components.

A DEAE cellulose column was prepared according to the method described by Sober, et al. (1956). The packed column was equilibrated with 0.005M sodium phosphate buffer, pH 7.0 in a cold temperature of 4°C prior to filtering through the toxin solution. The sample to be chromatographed was initially dissolved in distilled water. The toxin solution was dialized overnight against a buffer solution which was similarly used to equilibrate the DEAE cellulose column.

The schedules for the various eluents employed in the gradient chromatography are listed in figure 1. In order to introduce a gradual change in pH and ionic strength to the charged column during the elution process, gradient elution was practiced. Effluents were collected in 5-6 ml fractions in tubes arranged in a fraction collector (Toyo Roshi Kaisha Ltd.), and placed in a refrigerator (4-5°C). Each effluent fraction collected was examined in a Beckman DU spectrophotometer; the optical densities at 260 and 280 milli-microns were read and recorded. The hydrogen ion concentrations of each fraction were measured with a Model G Beckman pH meter and recorded.

As shown in the chromatograms, figure 1A, the UV absorbing fractions began to appear in the latter half of the initially added volumn of partially purified toxin. The hydrogen ion concentration of the effluent fractions remained within the pH 7.0 range, which is the initial reaction of the column after equilibration. Optically dense fractions continued to appear throughout the elution with the addition of eluent I, but the pHs remained in the 7.1 range. The spectrophotometric examination of the series of effluent fractions collected after eluent II

was added showed no significant amount of UV absorbing material present. However, the pH curve showed a gradual change from 7.0 to 6.9. When the salt gradient was increased by the addition of eluent III, a gradual rise in pH to 7.2 was observed. In this pH range the elution of UV absorbing fractions became apparent (in effluent fractions #210 through #225). The pHs of the fractions began to drop just prior to the addition of eluent IV and gradually leveled to pH 6.45. Simultaneously with the pH drop, the optical densities of the effluent fractions increased (#260 through #310). After a gradual drop to pH 6.45, the pHs of the effluent fractions, beginning with tube #299, rose to 6.8.

The salt gradient was increased by the addition of eluent V, and a gradual drop in the pH to about 6.0 was noted in the succeeding 50 effluent fractions. The optical density began to show an increase in the effluent fractions, beginning with tube #336; the maximum peak was recorded in the fraction of tube #363. Following the addition of the proceeding two eluents, VI and VII, the pH of the effluent fractions began to stabilize at 5.9, but UV absorbing fractions appeared in tubes #420 through #480 of the VI series and in tubes #510 through #570 of the VII series.

Although the salt gradient was increased by the addition of eluent VIII, no appreciable changes in optical densities were noted in the effluent fractions; only a slight decrease to pH 5.8 occurred. This pH was maintained throughout the fractions collected in series VIII and part of IX. In tube #730 (IX series), both the optical densities and the pHs of the effluent fractions began to show an increase; the maximum peak was demonstrated by fraction #737 with a pH of 5.89, while fraction #742 registered a pH reading of 5.96 and an OD reading much lower than that of fraction #742.

The effluent fractions, as indicated in table 1, were tested against an anti-E serum prepared by the Wellcome Laboratories and an anti-iota serum prepared by the investigator, using the agar-gel diffusion method of Wilson and Pringle (1954). Table 1 shows the results obtained after testing 10 different fractions against two different antisera. According to the results shown in table 1, 5 different antigenic components were detected in the partially purified material using the investigator's anti-E serum, but only 2 could be detected using the Wellcome product. Figure 2 shows the precipitin patterns obtained when 2 antisera were tested against 10 effluent fractions listed in Table 1.

Summary and Conclusions:

Iota toxin was separated from a partially purified material on a DEAE cellulose column. The individual antigenic components can best be resolved through the establishment of a gradual increase in ionic strength to the charged column. At least 5 different antigenic components were obtained by using the DEAE cellulose chromatography. Investigation will be continued to obtain quanities of iota toxin for chemical analysis and for use as a diagnostic antigen.

CHROMATOGRAPHY OF PARTIALLY PURIFIED IOTA TOXIN ON DEAE CELLULOSE COLUMN.

(I) 0.005M Na_2HPO_4-NaH_2PO_4
(II) 0.010M Na_2HPO_4-NaH_2PO_4
(III) 0.050M Na_2HPO_4-NaH_2PO_4
(IV) 0.10M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4
(V) 0.15M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4
(VI) 0.20M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4
(VII) 0.25M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4
(VIII) 0.30M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4
(IX) 0.50M NaCl - 0.05 M Na_2HPO_4-NaH_2PO_4

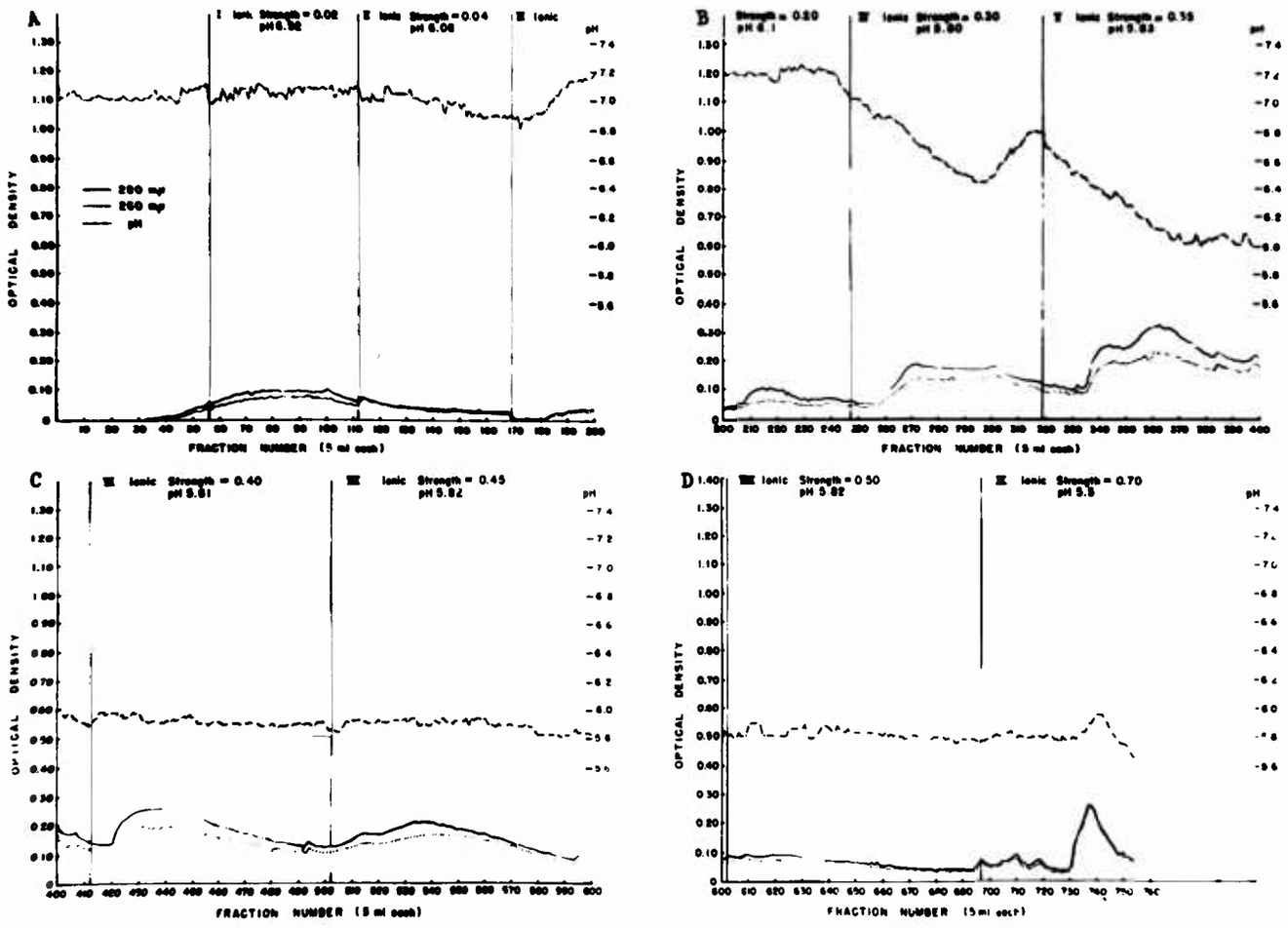


Table 1.

Patterns of Precipitin Reaction Obtained on Agar-Gel Plates

Fraction Number	Wellcome Serum	Prepared Serum	Remarks
100	-	-	1
217	-	/	1
272	/	/	1
300	-	/	2
344	-	/	2
363	/	/	3
433	-	/	3
439	-	/	4
517	-	/	4
534	-	/	
595	-	/	
737	-	-	

Legend:
(-) : No precipitin reaction
(/) : Positive precipitin reaction
(1) : Identical reaction; 217, 272 and 300
(2) : Identical reaction; 344 and 363
(3) : Identical reaction; 433 and 439
(4) : Identical reaction; 517 and 534

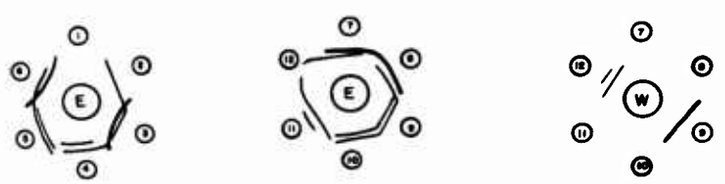


Figure —— Precipitin reactions on Agar-gelplates, 13 days after 37°C incubation.

(E) Anti-iota Rabbit serum
(W) Anti-E of Wellcome Research Laboratories

(1)	Fraction	# 100	(7)	Fraction	# 433
(2)	"	# 217	(8)	"	# 439
(3)	"	# 272	(9)	"	# 517
(4)	"	# 300	(10)	"	# 534
(5)	"	# 344	(11)	"	# 595
(6)	"	# 363	(12)	"	# 737

Figure 2

References:

1. Sober, H. A., Gutter, F. J., Wyckoff, M. M. and Peterson, E. A.: Chromatography of proteins. II. Fractionation of serum protein on anion-exchange cellulose. J. Am. Chem. Soc., 78: 756-763, 1956.

2. Wilson, M. W. and Pringle, B. H.: Experimental studies of the agar-plate precipitin test of Ouchterlony. J. Immunol., 73: 232-243, 1954.

CHEMISTRY DEPARTMENT

Technical activities of the Chemistry Department were performed within the following four functional areas: 1) diagnostic clinical chemistry, 2) analytical and water, 3) radioactivity analyses and 4) toxicology. Personnel of the Department performed numerous routine and miscellaneous analyses within each functional area for medical facilities in the WESTPAC area. These include military installations, United Nation laboratories and some civilian medical agencies. During the report period the following services were provided to these facilities by the Chemistry Department: (1) various essential analyses requiring reagents or equipment which were unavailable to them, (2) technical assistance in standardizing laboratory procedures, (3) non-standard essential items of supply on an emergency basis, and (4) consultation on special problems involving toxicology and clinical chemistry, or any other problems related to these fields.

During the period covered by this report there was an overall increase in work load of approximately 11.5 per cent in this department. This increase was partially due to a Free Iodine Study conducted for the Military Advisory Group in Korea. Details of this study will appear later in this report. In addition some work was performed on a special viral hepatitis project conducted by the Department of Virology and Rickettsial Diseases.

Analytical and Water Analyses

This section furnished support by providing a wide variety of complex analyses on many materials, such as locally procured pharmaceuticals, household chemicals and other commercial products both chemical and biological. Further, routine analyses of drinking water, water from new sources, sewage effluent and boiler water were provided in accordance with the mission of the Department. The total work performance of this section is listed in Table 1.

It is noteworthy that only 19 different procedures were reported last year as compared to 75 different procedures accomplished this year. It was necessary to apply new procedures due to increased demands for identification of varied materials and unusual drugs. Table 1 shows that 99 determinations were made of varied materials by this section.

Radioactivity Measurements

The Department has continued to monitor the atmosphere for radioactivity in the Zama area as well as performing radioactivity measurements on dust, water and foods received from various installations in WESTPAC.

The continuous monitoring of radioactive fallout was conducted and reported monthly. The following were methods employed:

1. A daily reading of 24-hour dust samples collected on a one-foot square gummed film obtained from the U. S. Atomic Energy Commission.

Table 1. Type and Number of Analytical and Water Analyses Performed.

Type	Number
Active chlorine determination	1
Alkalinity of water	97
Alkaloids in tablets	6
Antimony in water	99
Amyl nitrite assay	3
Assay for active chloride	8
Assay for dimercaprol	3
Assay for active iodine	80
Assay of water purification tablets	64
Calcium in water	77
Chloride in water	105
Color of water	69
Cyanides in water	56
Distillation of water	47
Ethanol qualitative determination	4
Examination of sodium lauryl sulfate	1
Extraction of oil in soil	1
Extraction of paper	1
Extraction of telvar	12
Extraction of toothpaste	1
Fluoride in water	106
Formaldehyde test	1
Gas chromatography for separation and identification of essential oil	3
Heavy metals tests	3
Hydrocarbons in water	1
Iron in injection	1
Iron in water	97
Isolation of secobarbital	1
Lead in serum	150
Lead in urine	491
Lead in water	1
Lime test	4
Magnesium in water	72
Manganese in water	73
Meperidine assay	2
Methanol test	3
Nitrate in water	125
Paper chromatographic test of alkaloids	7
Pentobarbital assay	1
pH of water	88
Phenols in water	7
Potassium in water	44

Table 1. (Cont'd)

Type	Number
Procaine tests	1
Silica in water	101
Silicate analysis	1
Sodium bicarbonate in tablets	2
Sodium carbonate tests	2
Sodium in water	44
Specificity tests	2
Sulfate in water	102
Test for acetone	1
Test for aldehydes	1
Test for antipyrine	1
Test for ascorbic acid	4
Test for aspirin	1
Test for chlorinated phenols	1
Test for fatty alcohol	1
Test for hydrogen peroxide	1
Test for photo developer	2
Test for oleic acid	1
Test for phenylpropanolamine	1
Test for phosphate and calcium	1
Test for silicones	1
Test for sodium hydroxide	1
Test of thyroid tablets	1
Total hardness of water	79
Total solids in fuel oil	16
Total solids in water	70
Turbidity of water	94
Ultraviolet spectral examination of phenylpropanolamine	1
USP tests	5
Whiskey analysis	1
2, 4-D test	1
Incineration of tooth paste	1
Infrared spectroscopic examination	217
Total	2,275

2. A weekly reading of total fallout collected by the water pot method using a glass jar (8 inches in diameter and 8 inches high).

3. A continuous automatic recording of radioactivity in filtered air by the use of a T-289 Air Sampler which operates on the principle of gas ionization.

4. Weekly readings of seven-day dust samples from Japan (Medical Laboratory, Yokosuka, Kuma), Ryukyu Islands (Okinawa, South Korea (Seoul and Pusan)),and South Vietnam (Hue).

5. Short term radioactivity monitoring of rain water and foods (leafy vegetables) which was initiated in September 1961 was continued on a more intensified basis. In order to complete this phase of radioactivity monitoring more effectively, a request for a research and development grant is being prepared for consideration.

Nuclear devices were detonated only during the months of August, September and December 1962. The highest peak of radioactivity fallout occurred during the month of October which was 14,000 micromicrocuries per square foot per day. This reading was still well below the level reached in November 1961. However, it is interesting to note that each peak of radioactivity fallout during the year was recorded approximately 48 hours after the particular device was detonated. This was evident particularly during the month of December 1962 when a series of detonations resulted in an increase in radioactive fallout. The levels were well below the 20 micromicrocuries per square foot per day on the 22nd and the 23rd of December, but on the 23rd, 24th and 25th three devices were detonated which resulted in a radioactivity fallout reading of approximately 30 micromicrocuries per square foot per day on the 24th and the 25th. The activity increased to 100 micromicrocuries per square foot per day and stayed at this level throughout the 24th, 25th, 26th and 27th. A series of detonations followed by rain fall of over 20 millimeters on the 29th, resulted in an increased fallout from 100 micromicrocuries per square foot per day to 5,860 micromicrocuries per square foot per day. On the 31st the levels returned to approximately 200 micromicrocuries per square foot per day and have continued to decrease during the year. Table 2 shows peaks of radioactivity fallout during the report period.

Dust Activity. The activity unit, $dpm/ft^2/day$ (disintegration per minute per square foot per day), for the radioactivity of dust and all other reports, except air sampling, was changed to $uuc/ft^2/day$ (micromicrocuries per square foot per day) in the month of October. In this way more accurate comparisons of results can be made with other agencies throughout the world who utilize the same unit of measurement. See Table 3. Radioactivity of filtered air will continue to be reported in millireps per hour. These results are calculated directly from the T-289 Air Sampler. (Figure 1).

Fallout Activity. Radioactivity of total fallout (rain and dust) collected by water pot method is listed in Table 4. The levels continued to decline during the first period of the year but as the amount of rain fall increased the total fallout increased proportionately. The highest levels were reached during the latter half of December. This sudden rise in fallout is attributed to the relatively high rain fall and increase in the number and/or types of nuclear devices detonated during November and December. With minor fluctuations the total fallout rate has continued to progressively decline throughout the remainder of the year. Present levels are approximately the same as they were at the beginning of the year.

Radioactive Monitoring of Filtered Air. Air samples were monitored 24 hours per day, 7 days per week by a T-289 Air Sampler system. Monitoring results were read directly from a recorded tape and calculator as total radioactivity instead of gamma and beta radiation. Monthly averages are shown in Table 5 which are reported in millireps per hour. The air activity rate continued to decline steadily from the beginning of the fiscal year, from approximately 200 millireps per hour to approximately 100 millireps per hour in January. The second peak was reached in May to approximately 160 millireps per hour and did not reach the maximum for the year during the month of July. Air activity has shown a continued decline. (Figure 2).

MONTHLY AVERAGE OF DUST ACTIVITY IN $\mu\mu c/ft^2/day$

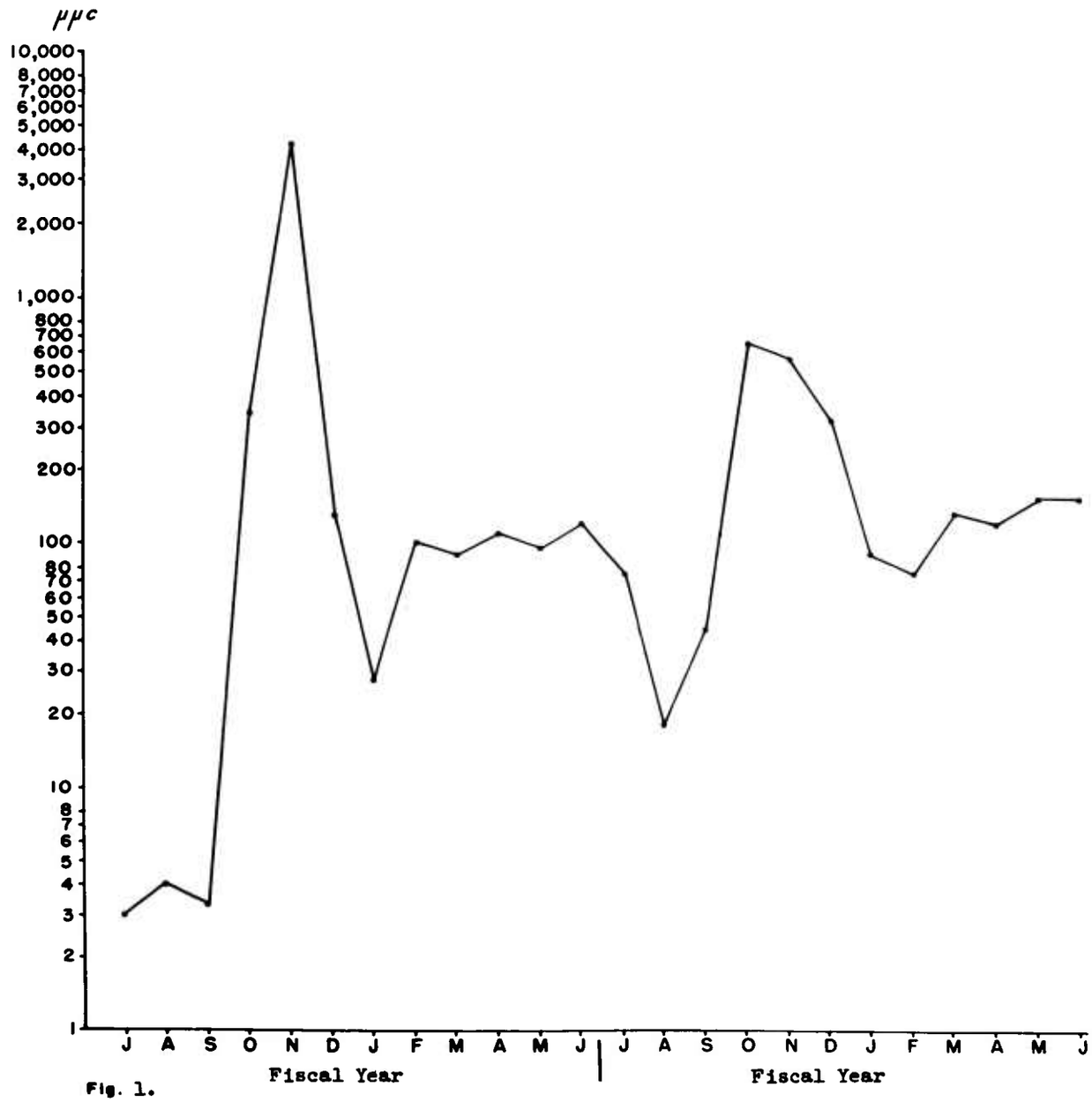


Fig. 1.

MONTHLY AVERAGE OF AIR ACTIVITY IN mr/hr 10^3

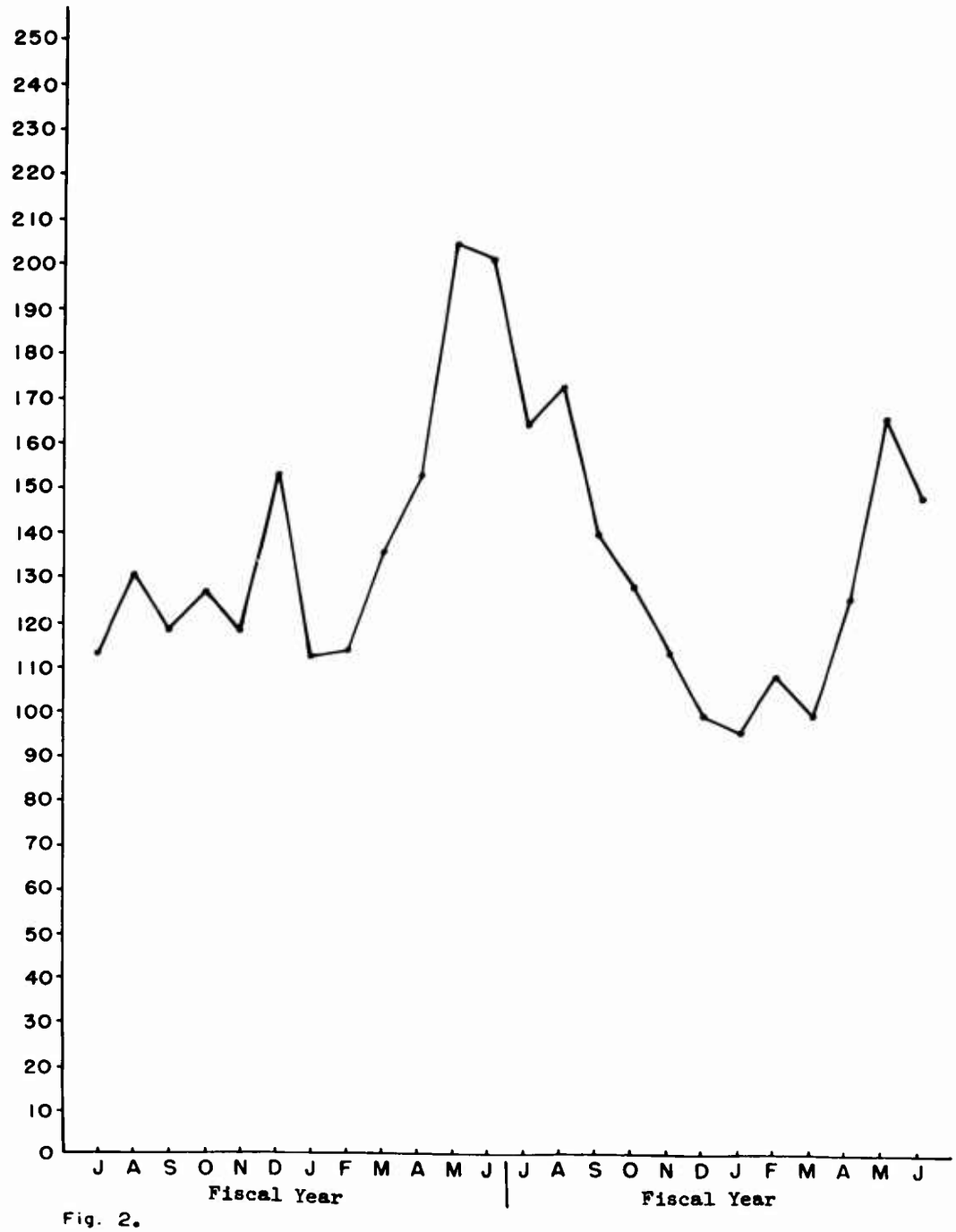


Fig. 2.

Table 2. Peaks of Radioactivity of Dust

Month	Date	Weather	Activity uuc/ft^2/day	Activity mc/mile^2/day
July	5-6	rain	169	4.73
1962	14-15	fair	299	8.40
Aug	22	fair	73	2.06
1962	23	fair	95	2.65
Sep	1	cloudy	112	3.16
1962	2	fair	112	3.16
	3	cloudy	112	3.16
	6	rain	119	3.34
Oct	15	rain	14,470	405.00
1962	22	rain	640	17.90
	28	rain	3,660	102.50
Nov	4	rain	4,050	113.00
1962	16-17	rain	2,055	57.50
	21-22	rain	2,810	78.60
Dec	5	rain	1,255	25.20
1962	30	rain	5,860	164.00
Jan	5	fair	378	10.60
1963	6	rain	378	10.60
Feb	12	fair	326	9.14
1963	26	rain	380	10.60
Mar	9-10	snow	400	11.20
1963	24	rain	1,210	33.90
Apr	7	rain	587	16.47
1963	15	rain	631	17.65
May	1	rain	736	20.58
1963				
Jun	3	rain	388	9.46
1963	6	rain	828	23.20
	13-14	rain	403	11.30

Table 3. Weekly and Monthly Averages of Dust Radioactivity

Month	Specimen No.	Collecting Period	Activity in uuc/ft^2/day
Jul 1962	258	26 Jun-2 Jul	42.0
	259	3 Jul-9 Jul	52.6
	260	10 Jul-16 Jul	144.0
	261	17 Jul-23 Jul	49.5
	262	24 Jul-30 Jul	4.2
	Monthly Average		58.4
Aug 1962	263	30 Jul-5 Aug	9.2
	264	6 Aug-12 Aug	9.4
	265	13 Aug-19 Aug	18.8
	266	20 Aug-26 Aug	157.2
	Monthly Average		37.2
Sep 1962	267	27 Aug-3 Sep	119.0
	268	4 Sep-10 Sep	38.6
	269	11 Sep-16 Sep	38.8
	270	17 Sep-23 Sep	22.6
	271	24 Sep-30 Sep	34.2
	Monthly Average		50.6
Oct 1962	272	1 Oct-7 Oct	34.2
	273	8 Oct-14 Oct	927.0
	274	15 Oct-21 Oct	121.0
	275	22 Oct-28 Oct	224.2
	Monthly Average		326.6
Nov 1962	276	29 Oct-4 Nov	217.6
	277	5 Nov-12 Nov	467.2
	278	13 Nov-18 Nov	207.4
	279	19 Nov-25 Nov	247.8
	Monthly Average		285.0
Dec 1962	280	26 Nov-2 Dec	318.6
	281	3 Dec-9 Dec	193.4
	282	10 Dec-16 Dec	80.0
	283	17 Dec-23 Dec	152.2
	284	24 Dec-30 Dec	270.2
	Monthly Average		202.9
Jan 1963	285	31 Dec-6 Jan	539.7
	286	7 Jan-13 Jan	309.1
	287	14 Jan-20 Jan	138.8
	288	21 Jan-27 Jan	118.0
	Monthly Average		276.4
Feb 1963	289	28 Jan-3 Feb	56.7
	290	4 Feb-10 Feb	59.0
	291	11 Feb-17 Feb	64.6
	292	18 Feb-24 Feb	32.6
	Monthly Average		53.2
Mar 1963	293	25 Feb-3 Mar	82.3
	294	4 Mar-10 Mar	99.8
	295	11 Mar-17 Mar	109.7
	296	18 Mar-24 Mar	119.7
	297	25 Mar-31 Mar	96.5
	Monthly Average		101.6
Apr 1963	298	1 Apr-7 Apr	201.4
	299	8 Apr-14 Apr	178.8
	300	15 Apr-21 Apr	126.8
	301	22 Apr-28 Apr	117.7
	Monthly Average		156.2
May 1963	302	29 Apr-5 May	142.0
	303	6 May-12 May	145.0
	304	13 May-19 May	89.5
	305	20 May-26 May	66.1
	Monthly Average		110.6
Jun 1963	306	27 May-2 Jun	82.0
	307	3 Jun-9 Jun	144.0
	308	10 Jun-16 Jun	107.0
	309	17 Jun-23 Jun	117.0
	310	24 Jun-30 Jun	34.0
	Monthly Average		97.0

Table 4. Weekly and Monthly Averages of Radioactivity of Total Fallout

Month	Specimen No.	Collecting Period	Activity uuc/ft^2/day	Activity mc/mile^2/day	Precipitation mm/week
Jul 1962	258	26 Jun - 2 Jul	105	2.93	71
	259	3 Jul - 9 Jul	66	1.85	69
	260	10 Jul - 16 Jul	165	4.62	24
	261	17 Jul - 23 Jul	382	1.89	27
	262	24 Jul - 29 Jul	38	1.07	42
		Monthly Average	88	2.47	47
Aug 1962	263	30 Jul - 5 Aug	12	0.34	0
	264	6 Aug - 12 Aug	10	0.28	0
	265	13 Aug - 17 Aug	8	0.21	0
	266	20 Aug - 24 Aug	24	0.68	3
		Monthly Average	14	0.38	1
Sep 1962	267	25 Aug - 3 Sep	85	2.37	0
	268	4 Sep - 10 Sep	22	0.60	1
	269	11 Sep - 16 Sep	13	0.36	0
	270	17 Sep - 23 Sep	7	0.21	0
	271	24 Sep - 30 Sep	5	1.35	0
		Monthly Average	35	0.98	0
Oct 1962	272	1 Oct - 7 Oct	23	0.65	6
	273	8 Oct - 15 Oct	555	15.50	24
	274	16 Oct - 21 Oct	351	9.82	1
	275	22 Oct - 28 Oct	704	20.15	35
		Monthly Average	408	11.53	17
Nov 1962	276	29 Oct - 4 Nov	170	4.76	110
	277	5 Nov - 12 Nov	160	4.48	0
	278	13 Nov - 18 Nov	453	12.60	40
	279	19 Nov - 25 Nov	675	18.90	15
		Monthly Average	365	10.18	41
Dec 1962	280	26 Nov - 2 Dec	286	8.02	32
	281	3 Dec - 9 Dec	194	5.41	5
	282	10 Dec - 16 Dec	90	2.52	4
	283	17 Dec - 23 Dec	27	0.74	-
	284	24 Dec - 30 Dec	1610	45.20	65
		Monthly Average	441	12.38	21
Jan 1963	285	31 Dec - 6 Jan	-	-	0
	286	7 Jan - 13 Jan	51	1.41	0
	287	14 Jan - 20 Jan	88	2.36	0
	288	21 Jan - 27 Jan	9	0.26	0
		Monthly Average	49	1.34	0

Table 4 (Cont'd)

Month	Specimen No.	Collecting Period	Activity uuc/ft^2/day	Activity mc/mile^2/day	Precipitation mm/week
Feb	289	28 Jan - 3 Feb	63	1.77	0
1963	290	4 Feb - 10 Feb	69	1.93	15
	291	11 Feb - 17 Feb	54	1.52	0
	292	18 Feb - 24 Feb	30	0.82	0
		Monthly Average	54	1.51	4
Mar	293	25 Feb - 3 Mar	202	5.64	5
1962	294	4 Mar - 10 Mar	182	5.10	10
	295	11 Mar - 17 Mar	258	7.21	44
	296	18 Mar - 24 Mar	273	7.66	28
	297	25 Mar - 31 Mar	77	2.17	3
		Monthly Average	198	5.55	18
Apr	298	1 Apr - 7 Apr	-	-	17
1963	299	8 Apr - 14 Apr	188	5.30	18
	300	15 Apr - 21 Apr	126	3.52	14
	301	22 Apr - 28 Apr	86	2.40	20
		Monthly Average	133	3.74	17
May	302	29 Apr - 5 May	252	7.02	30
1963	303	6 May - 12 May	115	2.87	12
	304	13 May - 19 May	313	8.75	47
	305	20 May - 26 May	92	2.52	5
		Monthly Average	193	5.29	24
Jun	306	27 May - 2 Jun	172	4.86	13
1963	307	3 Jun - 9 Jun	471	13.20	224
	308	10 Jun - 16 Jun	167	4.68	38
	309	17 Jun - 23 Jun	57	1.59	0
	310	27 Jun - 30 Jun	47	1.31	0
		Monthly Average	183	5.12	92

Table 5. Monthly Average of Filtered Air Activity

Month	Year	Air Activity in millirep per hour
Jul	1962	0.164
Aug	1962	0.172
Sep	1962	0.139
Oct	1962	0.127
Nov	1962	0.113
Dec	1962	0.089
Jan	1963	0.083
Feb	1963	0.108
Mar	1963	0.089
Apr	1963	0.123
May	1963	0.165
Jun	1963	0.127

Table 6. Weekly and Monthly Averages of Radioactivity of Dust Collected in Japan, Ryukyu Islands, S. Korea and S. Vietnam

Month	Sample No.	Collecting Period	Activity in uuc/ft^2/day						
			Japan			Ryukyu	Korea		S. Viet
			Kuma	Zama	Yokosuka	Okinawa	Seoul	Pusan	Hue
Jul	258	26 Jun - 2 Jul		87		44		33	7
1962	259	3 Jul - 9 Jul		74		-		82	2
	260	10 Jul - 16 Jul		203		0		231	8
	261	17 Jul - 23 Jul		111		6		75	6
	262	24 Jul - 29 Jul		12		2		1	2
		Monthly Average		97		13		84	5
Aug	263	30 Jul - 5 Aug		9		-	12	10	6
1962	264	6 Aug - 12 Aug		6		2	12	22	5
	265	13 Aug - 19 Aug		10		16	33	13	22
	266	20 Aug - 26 Aug		27		-	402	73	27
		Monthly Average		13		44	114	30	15
Sep	267	27 Aug - 3 Sep		61		6	308	211	12
1962	268	4 Sep - 10 Sep		25		5	23	32	108
	269	11 Sep - 16 Sep		7		14	80	76	17
	270	17 Sep - 23 Sep		7		18	-	43	-
	271	24 Sep - 30 Sep		34		72	-	9	22
		Monthly Average		27		23	137	74	40
Oct	272	1 Oct - 7 Oct		38		46	-		21
1962	273	8 Oct - 14 Oct		2518		63	-	32	17
	274	15 Oct - 21 Oct		97		210	-	1110	161
	275	22 Oct - 28 Oct		594		322	16	16	115
		Monthly Average		812		160	16	75	79
Nov	276	29 Oct - 4 Nov		306		113	175	153	341
1962	277	5 Nov - 12 Nov		94		166	736	40	1300
	278	13 Nov - 18 Nov		683		160	150	25	19
	279	19 Nov - 25 Nov		153		643	210	186	47
		Monthly Average		309		271	318	101	427
Dec	280	26 Nov - 2 Dec		231		412	136	64	750
1962	281	3 Dec - 9 Dec		150		232	165	49	371
	282	10 Dec - 16 Dec		60		112	53	95	80
	283	17 Dec - 23 Dec		27		79	381	33	241
	284	24 Dec - 30 Dec		598		464	75	101	113
		Monthly Average		213		260	162	68	311
Jan	285	31 Dec - 6 Jan		118	93	2010	75	190	753
1963	286	7 Jan - 13 Jan		17	9	985	21	68	755
	287	14 Jan - 20 Jan		37	59	564	45	50	78
	288	21 Jan - 27 Jan		11	9	254	8	-	308
		Monthly Average		46	42	953	37	103	474

Table 6 (Cont'd)

Month	Sample No.	Collecting Period	Activity in uuc/ft^2/day						
			Japan			Ryukyu	Korea		S. Viet
			Kuma	Zama	Yokosuka	Okinawa	Seoul	Pusan	Hue
Feb									
1963	289	28 Jan - 3 Feb	24	65	82	99	15	12	100
	290	4 Feb - 10 Feb	25	30	50	207	11	12	78
	291	11 Feb - 17 Feb	30	68	34	268	9	25	18
	292	18 Feb - 24 Feb	61	18	25	59	6	-	27
		Monthly Average	35	45	48	158	10	16	56
Mar	293	25 Feb - 3 Mar	29	68	27	84	123	53	189
1963	294	4 Mar - 10 Mar	30	164	79	170	70	-	86
	295	11 Mar - 17 Mar	60	261	58	165	108	54	62
	296	18 Mar - 24 Mar	-	191	66	321	93	30	18
	297	25 Mar - 31 Mar	-	52	14	66	88	283	76
		Monthly Average	40	147	49	161	96	105	86
Apr	298	1 Apr - 7 Apr	319	103	61	68	247	376	236
1963	299	8 Apr - 14 Apr	76	88	60	123	299	592	14
	300	15 Apr - 21 Apr	242	138	127	24	185	170	22
	301	22 Apr - 28 Apr	93	61	105	32	149	370	14
		Monthly Average	182	98	88	62	220	377	72
May	302	29 Apr - 5 May	56	185	167	29	45	370	-
1963	303	6 May - 12 May	227	128	160	74	75	206	-
	304	13 May - 19 May	141	234	191	6	113	123	13
	305	20 May - 26 May	103	68	45	13	149	51	34
		Monthly Average	132	154	141	31	96	188	27
Jun	306	27 May - 2 Jun	103	108	133	33	59	123	16
1963	307	3 Jun - 9 Jun	93	340	296	51	151	54	25
	308	10 Jun - 16 Jun	98	193	162	110	-	68	13
	309	17 Jun - 23 Jun	209	59	76	-	103	139	-
	310	24 Jun - 30 Jun	-	43	25	-	-	-	-
		Monthly Average	125	149	138	65	88	96	18

Table 7. Radioactivity of Rain Water

Month	Raining Period (Date)		Precipitation in mm		Radioactivity mc/mile^2		Radioactivity uuc/ml	
	Zama	Yokosuka	Zama	Yokosuka	Zama	Yokosuka	Zama	Yokosuka
Jul 1962	1-2		51		29.4		0.22	
	4		2		4.0		0.34	
	5-6		5		4.2		0.23	
	8-9		62		4.6		0.03	
	13		24		43.6		0.72	
	18		25		36.8		0.66	
	19-20		2		0.5		0.09	
	24		1		1.7		1.41	
	28		42		4.2		0.04	
Aug 1962	20		3		2.0		0.28	
Sep 1962	4		1		9.5		0.04	
	7		1		4.5		0.35	
Oct 1962	4		6		19.6		1.12	
	11		20		3.4		0.70	
	15		4		224.0		20.10	
	21		1		43.7		120.00	
	28-29		70		161.0		0.95	
Nov 1962	3-4		75		174.0		0.94	
	14-17		40		222.0		2.22	
	21-22		15		202.0		5.05	
	26		25		20.0		0.35	
	28		7		28.0		1.35	
Dec 1962	2		1		5.0		6.30	
	5		5		27.9		1.39	
	15		4		11.6		1.22	
	30		65		309.5		1.93	
Jan 1963	5		0.2		17.0		58.28	
Feb 1963	8		15		20.4		0.60	
	26		5		30.3		2.42	
Mar 1963	6	6	2*	5*	17.1	17.1	3.90	0.88
	9-10	8-9	9*	20*	14.3	21.8	0.60	0.89
	13	15-16	16*	-	22.6	-	0.54	-
	16	24	27	45	36.6	37.6	0.51	0.42
	24	28	28	5	84.5	4.7	0.11	0.79
	28-29		3		7.8		0.97	

Table 7. (Cont'd)

Month	Raining Period (Date)		Precipitation in mm		Radioactivity mc/mile^2		Radioactivity uuc/ml	
	Zama	Yokosuka	Zama	Yokosuka	Zama	Yokosuka	Zama	Yokosuka
Apr	7-8	7-8	35	28	85.7	25.6	0.88	0.60
1963	15-16	15	14	2	41.6	17.5	1.10	5.66
	23-25	17	20	8	21.9	19.4	0.42	1.01
		19		24		9.3		0.27
		23-24		36		46.7		0.58
		25		2		17.9		0.01
May	5-6	1	8	39	25.1	29.8	1.25	0.36
1963	9	5-6	4	41	14.7	39.1	1.15	0.47
	15-18	8-9	47	36	69.4	43.7	0.59	0.58
	20-21	11	5	25	10.8	21.6	0.75	0.57
	28	15(A)	13	4	12.0	13.0	0.36	2.65
		15(B)		20		12.0		0.41
		16-17		65		60.5		0.43
		20-21		13		22.4		0.80
		27-28		45		47.6		0.47
		29		5		5.0		0.95
		31		6		8.9		1.30
Jun	28 May							
1963	-5 Jun	2-4	168	83	29.0	56.2	0.07	0.29
	6-8	5	56	103	60.7	19.5	0.44	0.08
	11-12	6-7	6	83	12.6	161.2	0.79	0.82
	13-14	11-12	32	15	26.2	42.3	0.33	1.27
		13		56		66.0		0.50
		14		2		12.1		4.31
		16		3		28.1		6.42

*Represents fractionation from snow

Radioactive Monitoring of Vegetables. This department resumed examination of vegetables in March 1963 for the following reasons: (1) to obtain good base line studies for a proposed research and development project; and (2) to accumulate data for future comparison. To date, 34 different vegetables have been examined. This report ends 30 June 1963, but data compiled in July are included since the only significant radioactive findings were obtained during this month. Readings on chives were the most significant results obtained in radioactive monitoring of vegetables. The pulp of these chives (Sample No. 27) contained 34.14 micromicrocuries per gram residue gamma radiation and 12.86 micromicrocuries per gram residue beta radiation. Sample No. 34 (also chives) gave a zero reading residue gamma radiation and 1.43 micromicrocuries per gram residue beta radiation.

Data presented are rather scant since these activities were suspended for approximately 9 months during the report period. It is planned to conduct extensive studies in this area and a more detailed report will follow next year. Table 8 shows the results of radiological examination of vegetables.

Table 8. Radiological Examination of Vegetables

			Activity in uuc/gm			
			Juice		Pulp	
No.	Date Rec'd	Specimen	Gamma	Beta	Gamma	Beta
1	13 Mar 63	Carrot	0	0.08	0	0.35
2	13 Mar 63	Bog-rhubarb	0	1.09	0	0.63
3	13 Mar 63	White radish	0.32	0	0	0.92
4	13 Mar 63	White radish	0	0	0	0.24
5	19 Mar 63	Spinach	0.54	0	2.27	1.56
6	13 Mar 63	White radish	0.18	0	0.15	0
7	20 Mar 63	Lotus root	0.72	0.61	0	0
8	20 Mar 63	Cabbage	0.18	0	0	0
9	18 Apr 63	Tomato	0	0.40	0.05	0.36
10	18 Apr 63	Radish	0	0.75	0	1.44
11	18 Apr 63	Radish	0	0.36	0	0.32
12	18 Apr 63	Carrot	0	0.36	0	0
13	18 Apr 63	Carrot	0	0.40	0	0
14	18 Apr 63	Carrot	0.67	0	0.55	0
15	18 Apr 63	Carrot	0.20	0	3.00	0
16	18 Apr 63	Welsh onion	0	0	0	0
17	18 Apr 63	Welsh onion	0	0	1.29	0.68
18	18 Apr 63	Welsh onion	0	0	0.79	0
19	18 Apr 63	Celery	0.13	0.12	0.53	0.75
20	18 Apr 63	Celery	0.28	0.09	0.32	0.35
21	18 Apr 63	Carrot	0.27	0	0.36	0.47
22	18 Apr 63	Radish	0.18	0	0.08	0.50
23	18 Apr 63	Cabbage	0.16	0.05	0.41	0.59
24	18 Apr 63	Cabbage	0.27	0	0.22	0.43
25	18 Apr 63	Cabbage	0.16	0	0.26	0.43
26	10 Jun 63	Green onion			1.19	0.34
27	17 Jun 63	Chives			34.14	12.86

Table 8 (Cont'd)

No.	Date	Specimen	Activity in uuc/gm			
			Juice		Pulp	
			Gamma	Beta	Gamma	Beta
28	10 Jun 63	Green onion			2.34	0.47
29	10 Jun 63	Green onion			0.96	0.77
30	17 Jun 63	Chinese cabbage			0.81	0.99
31	1 Jul 63	White cabbage			0.41	0.94
32	1 Jul 63	Radish			1.67	0.91
33	1 Jul 63	Chinese cabbage			0	0.59
34	1 Jul 63	Chives			0	1.43

Toxicology

This section continues to provide toxicological analyses to facilities of the U. S. Security Forces in the WESTPAC area. Since this is the only major toxicology facility (primarily for biological specimens) in this area, many types of procedures are requested. Pharmaceutical identification of substances in biological fluids and tissues is a major part of the toxicological service. The type and number of procedures performed are listed in Table 9.

Of 548 cases submitted to this section during the period covered by this report, 56 autopsy cases were submitted by the Department of Pathology for toxicological analyses. Findings in several of these cases in which drugs or toxic agents were significant in the cause of death are listed in Table 10.

High ethanol content was found in blood specimens from 4 autopsy cases, and in 2 of these cases high volatile reducing substances were also found in tissue specimens. Five cases in which carbon monoxide poisoning was suspected as cause of death were submitted. Toxicological analyses confirmed the presence of carbon monoxide in significant amounts in all these cases. In one case a man was found badly burned in a house fire, but the blood specimen (heart) failed to contain any trace of carbon monoxide.

Four known suicide cases were submitted for confirmation of the causes of death. In one case a young female ingested Darvon (d-Propoxyhpene hydrochloride) which is commercially available. Stomach contents, kidney, and liver were strongly positive for Darvon. Another case was Dilantin (diphenylhydantoin) ingestion. Dilantin was found in early pure form in the stomach contents but tissue findings were negative. Barbiturates, however, were found in stomach contents - 7.6 mg per cent, liver - 1.4 mg per cent, kidney - 0.6 mg per cent, brain - 0.5 mg per cent, and heart - 0.4 mg per cent. In two other cases, shown as A and B in Table 11, a very significant amount of chloroquine was detected in all tissues.

A blood alcohol determination is routinely performed on all military individuals involved in automobile accidents. One hundred forty-nine blood alcohol cases were submitted and 40 showed alcohol levels greater than 1.5 mg/ml of blood, which, according to the National Safety Committee 1932, is considered to be indicative of intoxication.

This section also continued to assist the various branches of the military services in detecting illegal users of narcotics in this theater. Sensitive analytical procedures are performed to detect opium alkaloids in the urine of suspected users. Fifty-seven urine specimens were evaluated for the presence of opium alkaloids with no positive morphine determination.

Two hundred and one specimens from 53 autopsy cases were analyzed for the presence of opium alkaloids, morphine and morphine derivatives, and codeine. Two cases were found to contain significant amounts of these drugs.

Table 9. Type and Number of Analytical Toxicology Procedures Performed

Type	No.	Type	No.
Acetone	351	Glutethimide (doriden)	64
Aldehyde, general & specific	508	Heavy metals, qualitative	220
Aminopyrine	34	Hydrocarbons, general	1
Amphetamine	39	Hyminal	2
Arsenic	186	Insecticides	6
Barbiturates, quantitative	765	Insect repellent	1
Benadryl	1	Iron	6
Benzene derivatives	2	Isopropanol, qualitative	257
Bromides, organic	49	Kerosene and oils	1
Calcium hypochlorite	1	Meprobamate	79
Camphor	1	Mercury	2
Carbon monoxide, quantitative	52	Methanol, qualitative & quantitative	268
Chlorbrommethane	1	Molybdenum	4
Chloride	1	Morphine, tissue & urine	354
Chlorinated hydrocarbons	257	Nalline	1
Chlorpromazine	7	Nicotine	7
Chloroquine, qualitative & quantitative	137	Nitrites	3
Chlortrimeton	7	Paragoric	1
Codeine, tissue & urine	346	Phenols & Cresols	254
Compazine	5	Phenothiazine	2
Coumarin	1	Physostigmine	3
Cutex nitrocellulose	1	Poly-hydroxy-alcohols	4
Cyanide, qualitative	267	Primaquine	1
Darvon	3	Pyrethrum	1
Dicoumarol	1	Quinoline	43
Diphenhydramine	1	Rauwolfia Alkaloids	3
Diphenylhydantoin	5	Salicylates, qualitative, quantitative	95
Ethanol, blood, quantitative	272	Sulfonamides	1
Ethanol, urine, quantitative	3	Strychnine	6
Ethanol, tissue, quantitative	290	Veratrum Alkaloids	3
Ethylene glycol	3	Warfarine	7
Fluorine	5	Zactirin	4
		Total	5,306

Table 10

A	Chloroquine - 633 micrograms/gm in kidney, 695 micrograms/gm in liver, 0.8 gm per cent in stomach content.
B	Chloroquine - 536.4 micrograms/gm in liver, 285 micrograms/gm in kidney, 174 micrograms/gm in lung.
C	Ethylene glycol in blood, stomach contents, liver, and kidney.
D	Seconal - 615.6 mg per cent in stomach content.
E	Darvon in stomach contents, liver, and kidney.
F	Dilantin and barbiturates in stomach contents.
G	Ethanol 380 mg per cent, methanol 5.4 mg per cent, and volatile reducing substances 1.366 mg per cent in stomach contents.
H	Ethanol 369 mg per cent, methanol 2.5 mg per cent, volatile reducing substances 2.36 gm per cent in stomach contents.
I	Volatile reducing substances 1.02 gm per cent, and the combination of phenothiogine, ETOH, and acetaldehyde in tissues.
J & K	Ethanol 410 mg per cent and 500 mg per cent in blood respectively.
L, M, N, O & Q	Carbon monoxide - 17.6 volume per cent, 9.5 volume per cent, 18.0 volume per cent, 13.6 volume per cent, and 15.1 volume per cent in blood respectively.

Table 11. Type and Number of Diagnostic Clinical Chemistry Procedures

Blood and Serum Type	No.	Blood and Serum Type	No.
Acid phosphatase	1	Fibrinogen	1
Alkaline phosphatase	8	Gamma globulin	7
Amylase	1	Glucose	27
Bilirubin	67	Hemoglobin	2
Bromsulfalein	4	Hemoglobin, electrophoresis	105
Calcium	19	Iodine, protein bound	3,906
Carotene	13	Iron	136
Cephalein flocculation	22	Iron binding capacity	127
Chloride	11	Isocitric dehydrogenase	1,203
Cholesterol, total and esters	41	Lipids, total	27
Copper	1	Lipoprotein, electrophoresis	9
Creatinine	4	Magnesium	2
Cryofibrinogen	1	Methemoglobin	2
Cryoglobulin, electrophoresis	4	Non-protein-nitrogen	1
Esterase (lipase)	37	Phosphorus	9
Fatty acid	1	Phospholipids	52

Table 11 (Cont'd)

Blood and Serum

Type	No.
Potassium	15
Protein fractionation, electrophoresis	472
Protein, total (A/G ratio)	498
Sodium	15
Sulfhemoglobin	1
Thymol turbidity	128
Transaminase, glutamic pyruvic	4,631
Transaminase, glutamic-oxalacetic	533
Triglyceride	3
Urea nitrogen	14
Uric acid	19
Vitamin A	4
Total	12,184

Urine

Type	
Acetic acid	1
Amino acid	4
Boric acid	1
Calcium, quantitative	3
Calculi, analysis of	11
Catecholamines	275
Chlorides	4
Coproporphyrins, quantitative	21
Creatine	2
Creatinine	2
Cystine	3
5-Hydroxy-3-indole acetic acid	38
17-Hydroxycorticosteroid	375
17-Ketosteroid	365
Magnesium	1
Myoglobin, amino acid	2
Nitrogen, amino acid	3
Non-protein nitrogen	10
Phenylpyruvic acid, qualitative & quantitative	9
Phosphorus	1
Porphobilinogen	6
Protein, quantitative	2
Sodium	1
Sugar, Benedict's	2
Sugar identification, chromatography	45
Urobilinogen, quantitative	5
Uroporphyrin, quantitative	18
Total	1,210

Feces

Type	No.
Bilirubin	1
Fat, quantitative	36
Starch, quantitative	1
Urobilin	2
Urobilinogen	10
Total	50

Spinal Fluid

Type	
Chloride	5
Glucose	5
Protein, pandy	6
Protein, quantitative	14
Protein, electrophoresis	5
Total	35

Miscellaneous

Type	
Glucose on monkey serum	14
Isocitric dehydrogenase on monkey serum	6
Iodine content in water	584
Protein, electrophoresis, on monkey serum	14
Protein, electrophoresis, on scorpion venom	69
Protein, electrophoresis, on snake venom	24
Protein, total, on scorpion venom	56
Transaminase, glutamic pyruvic	14
Transaminase, glutamic-oxalacetic	14
Total	795

TOTAL 14,274

Clinical Chemistry

Diagnostic clinical chemistry analyses were performed for all military medical facilities and some non-military medical agencies in the WESTPAC area upon request. The services provided include: 1) performing special analyses on biological materials which normally require equipment and reagents not available to small clinical laboratories, 2) acting as a referral laboratory for all special clinical requests, and 3) providing refresher courses in laboratory procedures and on-the-job training for new technicians. A summary of the wide spectrum of clinical chemistry performed is shown in Table 11.

Research and Development Activities: Procedures on monkey serum were done in a cooperative effort with an ongoing research project in the Department of Bacteriology. A series of electrophoretic patterns were performed on snake and scorpion venoms in conjunction with the Department of Entomology's research and development program.

New Procedures. Several old procedures were revised and additional new procedures were periodically introduced because of the frequency of requests for a particular determination. Currently used methods were also re-evaluated to make new improvements in technique and to utilize methods that were more efficient and reliable.

One of the most significant procedures adopted by this department during the year was the circular paper chromatography technique for determining glucose and triglyceride in serum and urine.

Several additional enzyme determinations have been implemented. It is planned to perform as many enzyme determinations as possible in order to render better service to supported installations which are neither staffed nor equipped to perform these procedures.

A Beckman GC-2A Model, gas chromatograph equipment with accessories, was received in May 1963. This instrument is primarily used for rapid separation, identification and quantitative determination of gases and relatively volatile liquids. Due to the difficulty in obtaining helium, nitrogen was used as a carrier gas. Prior to using the instrument for analysis of unknown mixtures, retention time data were determined for numerous liquids up to a boiling point of 210°C. This was done with a Silicone 550 chromatographic column under various conditions of carrier gas speed, column temperature and filament current of the thermal conductivity detector.

It was found that the retention time data are consistent for a given compound under similar conditions of carrier gas speed and column temperature. The manual, provided by the manufacturer of the equipment, recommended that a filament current of 200 mA be employed. However, with nitrogen as a carrier gas, and a filament current of 200 mA, many compounds such as alcohols, ketones, and ethers, showed negative responses. This was probably due to thermal degradation of compounds liberating hydrogen. Hydrogen has a higher thermal conductivity than nitrogen.

The gas chromatograph was successfully applied in the separation and identification of eucalyptol oil containing camphor, phenol and guaicol. It has also been successfully used in the detection of contaminating gases in oxygen tanks carried by skin divers. Thus far, attempts to separate methanol and fusel oil constituents in

Korean alcoholic beverages has proved to be unsuccessful. This is due to the strong tailing effect of the polar components (water, ethanol) which result from adsorption of these compounds to the filling material in the column. It has been determined that high sensitivity detectors such as those based on hydrogen flame ionization and heat-stable chromatographic columns will be necessary for analysis of higher boiling liquid and solid samples.

Technical Proficiency Survey. Quarterly technical proficiency surveys of military laboratories in Japan and in other WESTPAC areas were conducted. Survey specimens were sent to participating laboratories for various clinical chemistry determinations. Approximately twenty-six laboratories participated in the program including two civilian laboratories.

Approximately one-half of the laboratories returned the results of the survey; the remaining laboratories replied "Not Performed." On most procedures an error of 5 per cent is acceptable, however, any error greater than this value may indicate 1) faulty technique, 2) deteriorated reagents, 3) faulty electronic equipment, or 4) contaminated glassware.

During the month of February the Korean Military Advisory Group requested that this department devise a method to determine whether field troops were using iodine tablets in canteen water for purification purposes. It was necessary to establish a simple procedure requiring a minimum of laboratory equipment and technical interpretation of test results. Therefore, it was decided to apply the color comparison method using a set of permanent standards. The reagent selected was a saturated solution of soluable starch. This reagent was chosen because it can be easily obtained in the field and it also produces an intense blue color when dissolved in a solution containing free iodine. In addition, the color developed does not lose its intensity for a long period of time.

The following iodine standards were used in the initial testing program:

½ tablet (4.0 mg/liter)
1 tablet (8.0 mg/liter)
1½ tablet (12.0 mg/liter)
2 tablets (16.0 mg/liter)

In the first series of 230 specimens of water from canteens, none were found to contain amounts of free iodine. Quantitative analysis showed no significant amount of free iodine (less than 1.0 mg/liter). In the second series of 354 specimens, only 31 canteens contained from 1 mg/liter to 16 mg/liter (✓) of free iodine. These results were obtained by applying the field test as well as quantitative analysis methods.

No additional specimens have been received for analysis, therefore, it can be concluded that this field test is acceptable and is being applied to test iodine content in canteens of water used by field troops.

DEPARTMENT OF ENTOMOLOGY

During the period 1 July 1962 through 30 June 1963 service and research activities were continued in the Department of Entomology. At times it was difficult to draw a clear line of demarcation between activities which could be termed "service" and those classified as "research". Generally, service activities performed by personnel of this Department included: Identification of arthropods and reptiles submitted from other installations; technical advice on insect control problems; instruction of insect and rodent control personnel assigned to various USARJ installations; maintenance of a reference collection of medically important arthropods, other invertebrates, venomous snakes of Southeast Asia; and surveillance of mosquito control effectiveness on or near USARJ installations, with special attention focused on the population of the Japanese encephalitis vector, Culex tritaeniorhynchus.

Research activities included: Biology of the Japanese encephalitis vector, Culex tritaeniorhynchus; entomologic aspects of overwintering of Japanese encephalitis virus; illustration of medically important arthropods, coelenterates, and venomous snakes for professional investigators from various areas of the world; compilation of material for revision of a manual of ixodid ticks of Japan, Korea and the Ryukyu Islands; preparation of a monograph on medically important scorpions of the world; attempts (still in progress) to prepare a polyvalent scorpion antivenin for use in all areas of the world where scorpion sting is a public health problem; properties of scorpion and snake venoms; and screening of potency of commercially available antivenins for treatment of bites by Southeast Asia snakes.

Entomologic Studies on the Overwintering of Japanese Encephalitis Virus

During the past few years activities on this project have centered on collection of Culex tritaeniorhynchus females during the winter months from suitable habitats such as caves, sheds, brush and wood piles. The small number of specimens taken each year in these winter collections has raised doubts in some quarters as to whether such overwintering females actually serve to perpetuate JEV or whether, at least in the Tokyo area, they constitute the sole means of virus survival during the winter. On this basis, preliminary investigations have been made to determine possible involvement of blood-sucking leeches in the epidemiology of JEV. Work to date has demonstrated survival of Nakayama strain JEV in the aquatic, blood-sucking leech Hirudo nipponia, for periods up to 20 days in leeches fed on viremic chicks and held thereafter at 6°C. This virus survived for nine days in leeches similarly fed and held thereafter at 22-24°C. Leeches receiving virus through both routes were killed by freezing and stored at -70°C for subsequent trituration with sterile sand for IC inoculation into 22-24 day old white mice for demonstration of virus survival. Mice were sacrificed on the fifth or sixth post-inoculation day and HA titers were determined. Studies are underway to determine whether a significant multiplication of virus occurs in leeches; whether leeches can transmit the virus while feeding; and whether infected leeches occur in nature. Colonies of aquatic and land leeches from Japan and Korea have been established in the laboratory. Chickens and white rats have been utilized as hosts for nasal leeches collected in Kyushu.

Collections of Hibernating Adult Mosquitoes. Although hibernating females of Culex tritaeniorhynchus have been collected during the winter months in the Tokyo area, rarity of such specimens raises the possibility that JEV may overwinter in another host. During FY 63 only one adult (female) C. tritaeniorhynchus was found during the winter months in collections from caves, sheds, brush piles and other likely habitats. In all such collections C. pipiens and C. hayashii predominated. These collections are summarized in Table 1. Since C. tritaeniorhynchus females appeared in light trap collections in April, it is possible that these mosquitoes overwinter in this area in an undiscovered habitat as yet.

Table 1. Adult Mosquitoes Collected from Overwintering Sites November 1962 - April 1963

Species	Sex	Collections Per Month						Total
		Nov	Dec	Jan	Feb	Mar	Apr	
Anopheles sineroides	M							0
	F			1	1	2		4
Anopheles sinensis	M							0
	F			1	1	2	1	5
Anopheles lindesayi	M							0
	F					1		1
Anopheles kareicus	M							0
	F					1		1
Culex tritaeniorhynchus	M							0
	F	1						1
Culex sasai	M						1	1
	F			4	1	2	4	11
Culex pipiens	M	20	6	27		1		54
	F	226	100	1,711	1,028	323	40	3,428
Culex orientalis	M							0
	F	79	26	65	12	27	7	216
Culex hayashii	M			102	29	8	2	141
	F			411	578	432	217	1,638
Total	M	20	6	129	29	9	3	196
	F	306	126	2,193	1,621	790	269	5,305

Determination of the Role of Blood-Sucking Leeches in the Epidemiology of Japanese Encephalitis

The problem initially was to determine whether Japanese Encephalitis virus, ingested during blood-meals from viremic chicks, can survive and propagate in the aquatic leech Hirudo nipponia Whitman. If survival and propagation of virus in annelid host are demonstrated, it would be necessary to determine whether leeches can transmit the virus while feeding; whether infected leeches can be found in nature; and whether these or other leeches may be significant in the epidemiology of Japanese Encephalitis.

Specimens of the Japanese medicinal leech, Hirudo nipponia Whitman, were purchased from a drug store specializing in folk medicines. The original source of these specimens could not be determined. Chicks and mice used in these studies were of laboratory stock. Tests were conducted with two lots of stock JEV (Nakayama Strain maintained in the Department of Virology of this Laboratory.

Leeches were maintained in the laboratory in plastic jars filled with water to a depth of four or five inches. The tops of the jars were secured with perforated plastic covers. It was necessary to line these jars with silk netting to prevent escape of the leeches. Leeches were not fed prior to their first blood-meal on viremic chicks. Water in the leech jars was changed twice weekly.

One-day old chicks were inoculated IV with 0.1 ml 10^{-3} Nakayama Strain Japanese Encephalitis virus. On the third day following inoculation the infected chicks were bled. A volume of 0.01 ml of blood was inoculated IC into each of ten 22-24 day old white mice. On the same day leeches were allowed to feed on these viremic chicks.

Chicks on which leeches were fed were restrained in wide-mouthed plastic cups in about two and one-half inches of water. Leeches dropped into these containers usually started feeding within a few minutes, and engorged in 8-12 minutes. In most instances two leeches were fed simultaneously on the same chick. It was estimated that a large example of H. nipponia might take as much as one ml of blood during engorgement.

Leeches engorged on the same chick were held together at room temperature, or in a refrigerator at 6°C in a large test tube partially filled with water and plugged with a cork pierced with a 19 gauge needle for ventilation. Water in the tube was changed every other day.

At 24 hour intervals following engorgement leeches were killed by quick-freezing with alcohol and dry ice and stored at -70°C. Because of the tough, rubbery consistency of leech tissues, it became necessary to prepare leeches for mouse inoculation by trituration with sterile sand. On one occasion three leeches were pooled to form material for an inoculum; in all other tests only two leeches were triturated together for this purpose.

The general plan of these preliminary tests was to determine survival of virus by inoculation into mice of material from leeches killed immediately and at 24 hour intervals after engorgement on viremic chicks. Control inoculations of pools of unfed leeches were made with each test, and evidence of viremia in chicks was obtained by inoculation into mice of chick blood taken on the same day on which the leeches were fed. Leeches inoculated with JEV received an inoculum of 0.1 ml 10^{-2} virus at a point on the dorsum approximately midway on the length of the annelid. Because of the extreme contractability of leeches the exact spot of inoculation could not be ascertained later. Also, it could not be established if the inoculum was injected into the gut of the leech or was deposited in botryoidal, muscular, or connective tissue.

Results of tests conducted to date are given in Tables 2 through 5. It was found that JEV survived for three or more days in approximately 50 per cent of the leeches fed on viremic chicks, and in 60 per cent of the leeches inoculated with virus. Maximum survival time for virus in leeches which had been fed on viremic chicks and held thereafter in a refrigerator at 6°C was 20 days (this is the maximum period tested). Virus survived for nine days in leeches fed in the same manner and held at 22-24°C.

Table 2. Results of Mouse Inoculation Tests to Determine Period of Survival of Japanese Encephalitis Virus in Leeches, Hirudo nipponia, fed on Viremic Chicks*

Period between feeding and freezing of leech	Tests with JEV lot prepared on 5 Sep 1961 No. of leeches in inoculum	Positive** results	Negative results	NA titer
Immediately	2	X		1:5120
One day	2	X		1:1280
Two days	2	X		1:2560
Three days	2	X		1:5120

* Leeches held at 22-24°C

** As evidence by illness or death of mice

Table 3. Results of Mouse Inoculation Tests to Determine Period of Survival of Japanese Encephalitis Virus in Leeches, Hirudo nipponia, fed on Viremic Chicks*

Period between feeding and freezing of leech	Tests with JEV lot prepared on 9 Apr 1963 No. of leeches in inoculum	Positive** results	Negative results	NA titers
Immediate (engorgement interrupted)	2	X		1:2560
Immediate (engorgement interrupted)	2	X		1:5120
One day	2	X		1:2560
Two days	2	X		1:1280
Three days (leeches had died overnight)	2		X	
Three days	2	X		1:320
Four days	2	X		1:2560
Four days	1		X	
Four days	2	X		1:320
Four days	2		X	

Table 3. (Cont'd)

Period between feeding and freezing of leech	Tests with JEV lot prepared on 9 Apr 1963 No. of leeches in inoculum	Positive** results	Negative results	NA titers
Five days	1		X	
Five days *	2	X		
Five days	2		X	
Six days	1		X	
Six days	2	X		1:320
Six days	2		X	
Seven days	2	X		1:320
Seven days	2		X	
Seven days	2		X	
Eight days	2		X	
Eight days	2	X		1:1280
Nine days	2	X		1:640
Nine days	2	X		1:640
Nine days	2		X	
Ten days	2		X	
Ten days	2		X	
Ten days	2		X	
Eleven days	2		X	
Eleven days	2		X	

* Leeches held at 22-24°C.

** As evidenced by illness or death of mice.

Table 4. Results of Mouse Inoculation Tests to Determine Period of Survival of Japanese Encephalitis Virus in Leeches, Hirudo nipponia, fed on Viremic Chicks*

Period between feeding and freezing of leech	Tests with JEV lot prepared on 9 Apr 1963 No. of leeches in inoculum	Positive** results	Negative results	HA titers
Three days	2	X		1:320
Four days	2	X		1:320
Five days	2	X		1:640
Six days	2	X		1:1280
Eight days	2		X	
Nine days	2		X	
Nine days	2	X		1:1280
Ten days	2		X	
Ten days	2	X		1:1280
Eleven days	2	X		1:640
Eleven days	2	X		1:640
Twelve days	2	X		1:1280
Thirteen days	2		X	
Fourteen days	2	X		1:2560
Fourteen days	2		X	
Fifteen days	2		X	
Fifteen days *	2	X		
Sixteen days	2		X	
Sixteen days	2		X	
Seventeen days	2	X		1:640
Seventeen days	2	X		1:1280

Table 4. (Cont'd)

Period between feeding and freezing of leech	Tests with JEV lot prepared on 9 Apr 1963 No. of leeches in inoculum	Positive** results	Negative results	HA titers
Eighteen days	2	X		1:1280
Twenty days	2	X		1:640

* Leeches held at 6°C.

** As evidenced by illness or death of mice.

Table 5. Results of Mouse Inoculation Tests to Determine Period of Survival of Japanese Encephalitis Virus Injected into Leeches, Hirudo nipponia*

Period between feeding and freezing of leech	Tests with JEV lot prepared on 9 Apr 1963 No. of leeches in inoculum	Positive** results	Negative results	HA titers
One day	1	X		1:1280
Two days	1	X		1:640
Three days	1	X		1:640
Four days	1		X	
Five days			X	

* Leeches held at 22-24°C.

** As evidenced by illness or death of mice.

Discussion: While the results obtained thus far in this study are rather erratic (perhaps due in part to the small numbers of leeches utilized), the fact remains that leeches constitute one of the few groups of blood-sucking invertebrates not previously investigated as reservoirs or vectors of arboviruses. This is rather surprising, as in some aspects of their biology, i.e., longevity, ability to survive without food for several months, and dependence on blood for food, leeches might well be compared with ticks. Moreover, blood-sucking leeches are widely distributed in areas where arboviruses are public health problems, and are known to feed on man, domestic animals, birds and other proven hosts of these viruses.

Hirudo nipponia Whitman, the leech used in these studies, like other members of the genus, is a blood-sucking species, and will feed on a variety of hosts including man, other mammals, birds, reptiles, and amphibians. In turn it may be eaten by birds and possibly other animals. Exact distribution of the species has not been determined, although it is said to occur throughout Japan. In the Tokyo area, at least, it is inactive during the winter and remains buried in mud and debris. Date of emergence and first activity in the spring in this area has not been reported. It is probable that this species is similar to other blood-sucking leeches in that digestion of blood meals is extremely slow and takes place over a period as long as six months. Specific digestive enzymes have not been found in blood-sucking leeches. It is thought that digestion of blood meals may be accomplished by gut-inhabiting bacteria of genus Pseudomonas. Japanese publications list three blood-sucking species of leeches from these islands. These are Hirudo nipponia, Whitmania pigra and the land leech Haemadipsa japonica. Detailed descriptions of habits, distribution and life cycles of these leeches have not been published.

As far as can be determined, the only published report of experimental work involving leeches and dealing with an arthropod-borne disease of man was that of Oshima, Asakura and Yoshii (1943). These workers described an experiment in which they allowed two lots of five leeches each(Hirudo nipponia) to feed on dengue patients. Leeches of one lot were subsequently held at room temperature (9-13°C) for 10 days then lyophilized and refrigerated. Leeches of the other lot were maintained at room temperature for 23 days before they were lyophilized. These lyophilized specimens were dissolved in saline and material from each lot was inoculated into a paretic female patient. Each patient received an inoculum of 0.2 ml in divided doses of 0.1 ml each on the outer surface of the upper arm. In each of these cases the patients were described as developing typical clinical dengue within six days in one instance, and seven in the other.

Additional studies are underway to determine duration of survival of JEV in leeches, to determine whether the virus can propagate in the leech, and whether leeches can transmit the agent while feeding. Later in the Summer attempts will be made to isolate JEV from wild-caught leeches from areas where JE is prevalent. Immediate attention is being given to establishment of leech colonies from Japan and adjacent areas. As a first step, a colony of approximately 1,000 specimens of H. nipponia from Kyushu is now established in the department. These specimens were collected by department personnel during June 1963. Living specimens were also collected in Korea during the latter part of June.

Reference: Oshima, Asakura and Yoshii. (1943). Primary studies on transmission of dengue fever with leech, Hirudo nipponica Whitman. Japanese Army Med. Sch. Epid. Rept. (in Japanese). Baeki-Kenkyu-Hokoku, Part II, No. 524:2-8.

Illustration of Medically Important Arthropods

The group of zoological artists at the Medical General Laboratory (406), is the only one of its kind in the Armed Services of the United States, and is the largest known unit of this type in existence. Since 1946 members of this group have produced a great variety of illustrations of medically important animals, with primary emphasis on species prevalent in Asia. These illustrations have been published in professional journals, military manuals, and monographs printed by private and governmental institutions. The earliest illustrations prepared by these artists were for monographs by military personnel on active duty, or by civilian scientists on contract with the Army. In recent years an ever-increasing percentage of the work load has been devoted to preparation of drawings for non-military agencies, of civilians with no direct contract with the Army. The criterion for acceptance of these projects depends on the medical importance of the species to be illustrated. This group of artists provides authors of professional stature with illustrations of a high quality at a very low cost. Principal investigators submit their material to the department, dissections, slides and pencilled drawings are accomplished as requested, the latter are checked for accuracy, and completed plates comprised of India-ink drawings are returned to the principal investigator together with all specimens.

The illustration of medically important arthropods continued at approximately the same productivity rate in FY 63 as in prior years. During the period covered by this report, one Supervisory Illustrator resigned and one Zoological Artist was trained. A total of 1,155 drawings of 138 species of medically important arthropods were completed during FY 63 as shown in Table 6.

Table 6. Number of Illustrations of Species Completed in FY 1963

Species	No. of illustrations	No. of species
Medically important scorpions of the world	59	14
Chiggers of South East Asia	10	1
Culicoides of South East Asia	89	13
Nearctic black flies	60	7
Ectoparasites of Panama (terminated in FY 63)	42	8
Studies on flies of medical importance in Japan	525	50
Ticks of Taiwan	59	8
Mosquitoes of Thailand	85	17
Black flies of Panama (terminated in FY 63)	226	20
Total	1,155	138

Medically Important Scorpions of the World. Principal investigator is Lt Colonel Hugh L. Keegan, MSC, Department of Entomology, Medical General Laboratory (406), U. S. Army Medical Command, Japan, APO 343, San Francisco, California.

Chiggers of South East Asia. Principal investigators are Colonel Robert Traub (Retired), Department of Microbiology, School of Medicine, University of Maryland, Baltimore 1, Maryland; Doctor J. Ralph Audy, The George Williams Hooper Foundation, University of California Medical Center, San Francisco, California; and Mr. M. Nadchatram, Medical Zoology Laboratory, Institute for Medical Research Kuala Lumpur, Malaya.

Culicoides of South East Asia. Principal investigator is Doctor Willis W. Wirth, U. S. Department of Agriculture, Washington 25, D. C.

Nearctic Black Flies. Principal investigator is Doctor Alan Stone, U. S. Department of Agriculture, Washington 25, D. C.

Ectoparasites of Panama. Principal investigator is Major Veron J. Tipton, MSC, Medical Field Service School, Brooke Army Medical Center, Fort Sam Houston, Texas.

Studies of Flies of Medical Importance in Japan. Principal investigators are Doctor Rokuro Kano, Department of Medical Zoology, Tokyo Medical and Dental University, Tokyo, Japan; and Lt Colonel Gordon Field, MSC, Army Environmental Health Agency, Edgewood Arsenal, Maryland.

Ticks of Taiwan. Principal investigators are Doctor Harry Hoogstraal, Naval Medical Research Unit No. 3, Cairo, Egypt; and Commander Robert Kuntz, National Naval Medical Center, Bethesda, Maryland.

Mosquitoes of Thailand. Principal investigator is Major John E. Scanlon, MSC, Department of Entomology, U. S. Component, SEATO Medical Research Laboratory, APO 146, San Francisco, California.

Black Flies of Panama. Principal investigator is Lt Colonel Gordon Field, MSC, Army Environmental Health Agency, Edgewood Arsenal, Maryland.

Studies on Flies of Medical Importance in Japan

This report covers the period 1 September 1962 - 30 June 1963. During this time, the contractor and his assistants performed eight field surveys; observed life cycles of fly species in the laboratory; wrote descriptions of new species and previously unrecorded species of sarcophagid flies; identified and examined fly specimens preserved in the laboratory; and observed distribution and seasonal occurrence of medically important flies in various prefectures throughout Japan. The results of these investigations are as follows:

Field Surveys: Six field surveys were conducted to investigate distribution and breeding places of medically important flies. Two surveys were made to determine if muscoid flies were vectors of thelaziasis.

Survey at Karuizawa, Nagano Prefecture: This survey was performed during 7-12 September 1962. Karuizawa is a mountainous area and is located in the central portion of Honshu. Karuizawa has many hills, forests, mountain streams and pastures. Many palaearctic species of insects, especially mountainous species, are prevalent in this area. The medically important flies collected during this survey consisted of seven families, 22 genera and 46 species numbering 2,764 specimens. A list of flies collected is shown in Table 7.

Four males and eight females of Phormia regina were obtained with a cage trap. This species was newly found in Karuizawa which became the new southern limit for the distribution of the species. This species was commonly found in Hokkaido. It was also found in Aomori, Niigata Prefectures, and rarely in Honshu. This finding is of epidemiological value since Phormia regina is a notoriously suspected vector of poliomyelitis and other infectious diseases.

Paired specimens of Sarcophaga takahasii were collected and additional useful data on the female of this species were obtained. A female of the S. kobayashii species was obtained at Sengataki, Nakakaruizawa, and brought back alive to the laboratory. This female deposited 16 first stage larvae on 8 September. The larvae became 2d instars on 9 September, 3d instars on the 10th, and became pupae on 17-19 September. Two males and five females emerged between 30 September and 2 October. All larval stages of S. kobayashii were found for the first time. Nine males and 16 females of Musca hervei emerged in the laboratory from cattle feces obtained at Oiwake.

Table 7. Medically Important Flies Collected at Karuizawa
7 - 12 September 1962

Family	Male	Female	Total
Muscidae			
Musca convexifrons	2		2
Musca domestica vicina	5	8	13
Musca hervei	9	16	25
Muscina angustifrons	2	8	10
Muscina pabulorum	2		2
Dasyphora syanicolor		1	1
Graphomyia maculata	1	1	2
Stomoxys calcitrans	13	18	31
Anthomyiidae			
Fannia scalaris	1	1	2
Ophyra leucostoma	1	6	7
Calliphoridae			
Aldrichina grahami	3	89	92
Chrysomya pinguis	280	426	706
Dexopollenia flava	10	6	16

Table 7 (Cont'd)

Family	Male	Female	Total
Isomyia senomera	103	194	297
Lucilia ampullacea	489	408	897
Lucilia caesar	489	408	897
Lucilia illustris	111	354	465
Lucilia papuensis		11	11
Melinda tsukamotoi	1		1
Melinda n. sp. 1	1	1	2
Melinda n. sp. 2	4		4
Phaenicia sericata	8	8	16
Phormia regina	4	8	12
Polleniopsis sp.	9	6	15
Stomorhina discolor	4	1	5
Triceratopyga calliphoroides		2	2
Sarcophagidae			
Sarcophaga albiceps	11	4	15
S. fieldi	2		2
S. harpax		1	1
S. hokurikuensis	1		1
S. kagaensis	6		6
S. kawayuensis	1	3	4
S. kobayashii	3	7	10
S. melanura	7	3	10
S. okazakii	1		1
S. peregrina	4	12	16
S. schutzei	3	2	5
S. septentrionalis	1	2	3
S. similis	14	28	42
S. takahasii	2	1	3
S. tsushimae	1		1
Syrphidae			
Tubifera tenax	1		1
Strathiomyiidae			
Ptecticus tenebrifer	2		2
Tabanidae			
Tabanus humilis		1	1
T. sapporaensis	1		1
T. rufidens		2	2
Total	1,124	1,640	2,764

Survey on Amami-Oshima Island and Kagoshima City, Kagoshima Prefecture: This survey was performed during 1 - 19 March 1963. The purpose of the survey was to conduct taxonomic and ecologic studies of medically important flies, particularly in their distribution and breeding places. Amami-Oshima is located in a subtropical area where many oriental species of insects can be found. Because of rainy weather during this survey, relatively few fly specimens were collected.

Medically important flies collected in the survey included representatives of five families, 16 genera and 22 species numbering 902 specimens (506 males, 393 females and 3 pupae). Lists of the flies taken are shown in Table 8 and 9.

One female of Synthesiomyia nudiseta was obtained at Shiroyama, Kagoshima City for the first time. This is a new record collection from the Japanese main islands (Hokkaido, Honshu, Shikoku and Kyushu). Shiroyama became the new northern limit for distribution of this fly.

Musca hervei, Lispe orientalis, Calliphora lata, Triceratopyga calliphoroides, Melinda sp. and Scopeuma mellipes were newly found on Amami-Oshima Island. As shown in Table 10, pupae of two sarcophagid species were found in a lavoratory of Yuwan, Amami-Oshima, and a pupa of Stomoxys calcitrans was found in a hogpen at Yuwan. These pupae were brought back alive to the laboratory and three female flies emerged from them.

Survey in the Ryukyu Islands (Okinawa Main Island, Miyako Island, Ishigaki Island and Iriomote Island).

This survey was performed during 18 April - 27 May 1963. The purpose of the survey was to investigate the distribution and breeding places of medically important flies. This is also a sub-tropical area and many oriental species of insects were found here.

Since good weather conditions prevailed during the survey a great many fly specimens were collected. These fly specimens included representatives of four families, 23 genera, 53 species numbering 2,642 specimens (1,485 males and 1,157 females). The list of the flies taken is shown in Table 10.

Most of the flies collected during this survey belong to the oriental species. Of these flies, six species are probably new and five species are newly recorded from Japan. The newly recorded species are as follows: Muscina angustifrons; Fannia prisca; Lucilia papuensis; Melinda itoi; and Bengalia bezzii.

Of the six new species, three new sarcophagid fly species were described by R. Kano and G. Field. This publication is now in press.

Most all maggots collected in lavatories on the Ryukyu Islands were Chrysomya megacerphala.

Table 8. Medically Important Flies Collected on Amami-Oshima and Kagoshima 1 - 19 March 1963

Family (Female F / Male M)	Locality: Kagoshima M	Kagoshima F	Nishinakama M	Nishinakama F	Mt. Yuwan M	Mt. Yuwan F	Uragami, Naze M	Uragami, Naze F	Naze City M	Naze City F	Total M	Total F	Grand Total
Muscidae													
Lispe orientalis								3				3	3
Musca domestica vicina	1	1		3				8		4	1	16	17
Musca hervei				2								2	2
Muscina angustifrons						1	1		1	9	2	10	12
Stomoxys calcitrans				3				11				14	14
Synthesimyia nudiseta		1										1	1
Anthomyiidae													
Fannia canicularis		1	15	1			50		16	1	81	3	84
Fannia prisca	1										1		1
Calliphoridae													
Aldrichina grahami	9	2									9	2	11
Calliphora lata	33	22	9	3	14	5	24	13	262	238	342	281	623
Chrysomya megacephala										1		1	1
Chryomya pinguis	1								2	23	3	23	26
Lucilia porphyrina			2	1	1			2	1	22	4	25	29
Melinda sp.			2								2		2
Phaenicia sericata										1		1	1
Triceratopyga calliphoroides	6	2							1		7	2	9
Sarcophagidae													
Goniophyto honshuensis				1			5	6			5	7	12
Sarcophage melanura	1						3	2	2		6	2	8
Sarcophaga oshimensis			6				2		2		10		10
Sarcophaga peregrina									1		1		1
Scatophagidae													
Scopeuma mellipes					32						32		32
Total	52	29	34	14	47	6	85	45	288	299	506	393	899

Table 9. Fly Pupae Collected at Yuwan, Amami-Oshima Island
7 March 1963

Species	Emerged adult	Breeding place
Stomoxys calcitrans	1 female	hogpen
Sarcophaga albiceps	1 female	lavatory
Sarcophaga melanura	1 female	lavatory

Table 10. Medically Important Flies Collected in the Ryukyu Islands
18 April - 27 May 1963

Family	Female F / Male M	Locality: Okinawa Main Island M	Okinawa Main Island F	Miyako Island M	Miyako Island F	Ishigaki Island M	Ishigaki Island F	Iriomote Island M	Iriomote Island F	Total M	Total F	Grand Total
Muscidae												
Graphomyia rufitibia						21	23		4	21	27	48
Morellia hortensia						2	8	10	86	12	94	106
Musca conducens				1	2	2	6	3	66	6	74	80
Musca domestica vicina		3	2	2	4		4	3	38	8	48	56
Musca gibsoni						2	1			2	1	3
Musca sorbens			1			1	4	13	28	14	33	47
Musca ventrosa				1				3		4		4
Muscina angustifrons		1	2							1	2	3
Orthellia sp. 1		4	4	4	9	4	14	80	139	92	166	258
Orthellia sp. 2		6	12		2	1		7	24	14	38	52
Orthellia sp. 3		1	9		6		1	6	21	7	37	44
Stomoxys calcitrans			4	12	11	5	8	18	19	35	42	77
Synthesiomyia nudiseta					1		1				2	2
Lispe orientalis		53	25	9		5	3	23	11	90	39	129
Anthomyiidae												
Anthomyia illocata			6			1		1	1	2	7	9
Fannia prisca		70	3							70	3	73
Ophyra chalcogaster		34	2	12	5	2	9	47	25	95	41	136
Calliphoridae												
Bengalia bezzii						2	1	5	1	7	2	9
Chrysomya megacephala		3	3	8	18	4	6	8	34	23	61	84
Chrysomya pinguis		3	4			21	19	1	1	25	24	49
Chrysomya rufifacies					1	1	2		3	1	6	7
Hemibyrellia ligurriens		3		5		3	4	9	1	20	5	25
Lucilia papuensis			1								1	1
Lucilia porphyrina			5			9	11	5	7	14	23	37

Table 10 (Cont'd)

Family	Locality: Okinawa Main Island M	F	Miyako Island M	F	Ishigaki Island M	F	Iriomote Island M	F	Total M	F	Grand Total
Melinda itoi	3								3		3
Phaenicia cuprina				1						1	1
Phaenicia sericata				1	1	1	18	22	19	24	43
Rhinia sp.							3	1	3	1	4
Stomorhina discolor		1	10	19	1			2	11	22	33
Stomorhina sp.							3		3		3
Strongyloneura sp.							5		5		5
Sarcophagidae											
Goniophyto honshuensis					1	1	4	1	5	2	7
Goniophyto sp.					30	20	6	2	36	22	58
Sarcophaga albiceps	6		7	14	13	16	9	10	35	40	75
Sarcophaga aniyai					36	3	8		44	3	47
Sarcophaga antilope	1				1				2		2
Sarcophaga basalis		1								1	1
Sarcophaga calicifera	1	2	1	2			2	1	4	5	9
Sarcophaga caudagalli			7	9			3		10	9	19
Sarcophaga fieldi	9	1			3		1		13	1	14
Sarcophaga josephi	28	6				1	22	8	50	15	65
Sarcophaga kinoshitai	2				1			1	3	1	4
Sarcophaga magensi					24	1	34	6	58	7	65
Sarcophaga melanura	3	4							3	4	7
Sarcophaga misera	16	7	36	20	26	5	29	20	107	52	159
Sarcophaga orchidea		1	22	24	11	7	29	13	62	45	107
Sarcophaga peregrina	63	23	44	22	29	18	63	16	199	79	278
Sarcophaga pseudo-subulata	37	6							37	6	43
Sarcophaga ruficornis			1	2					1	2	3
Sarcophaga shirakii	48	6							48	6	54
Sarcophaga tuberosa	3	1	6		1				10	1	11
Sarcophaga yonahansis	16	1							16	1	17
Sarcophila cinerea				1	3	5	138	19	141	25	166
Total	417	143	188	174	267	203	619	631	1,491	1,151	2,642

Survey on Amami-Oshima Island, Kagoshima Prefecture: This survey was performed mainly in Naze City and Mt. Yuwan during 12-27 June 1963. The purpose of the survey was to investigate filth flies, especially their distribution and breeding places.

In this survey, four families, 12 genera, 26 species numbering 346 flies were collected. The list of flies taken is shown in Table 11.

Due to good weather during the second survey conducted on Amami-Oshima Island, many fly specimens were obtained. Of these flies, two sarcophagid flies were new and Melinda tsukamotoi, Sarcophaga fieldi and Sarcophila cinerea were newly recorded. Two new species of sarcophagid flies were described by R. Kano and G. Field. This paper will be published in the near future.

Table 11. Medically Important Flies Collected on Amami-Oshima Island 12 - 27 June 1963

Family	Locality: Mt. Yuwan M	Mt. Yuwan F	To-jo, Sumiyo M	To-jo, Sumiyo F	Naze M	Naze F	Asani M	Asani F	Total M	Total F	Grand Total
Muscidae											
Musca hervei				1						1	1
Musca sorbens			1						1		1
Orthellia latipalpis		6								6	6
Anthomyiidae											
Ophyra chalcogaster		2				1		1		4	4
Ophyra leucostoma		1								1	1
Calliphoridae											
Lucilia porphyrina	2	9							2	9	11
Lucilia papuensis		1								1	1
Hemipyrellia ligurriens			1						1		1
Aldrichina grahami		3								3	3
Chrysomya pinguis	1	11							1	11	12
Melinda tsukamotoi	2	4							2	4	6
Stomorhina discolor				1						1	1
Sarcophagidae											
Sarcophaga peregrina	18	15	6	7	9	4	10	5	43	31	74
Sarcophaga albiceps		1	1	2			1		2	3	5
Sarcophaga josephi	58	1			5		5		63	6	69
Sarcophaga fieldi	11								11		11
Sarcophaga sp. 1	36	26	1		1		4		42	26	68
Sarcophaga sp. 2	49	3							49	3	52
Sarcophaga antilope	3	3							3	3	6
Sarcophaga misera			4						4		4
Sarcophaga melanura			3						3		3
Sarcophaga orchidea			1						1		1
Sarcophaga tuberosa			1						1		1
Sarcophaga calicifera			1						1		1
Sarcophila cinerea			1						1		1
Geniophyto honshuensis			1						1		1
Total	180	86	22	11	15	5	20	6	232	113	345

Survey in Shikoku District: This survey was performed in Kotohira, Kagawa Prefecture, and Omogo-kei, Ehime Prefecture during 24-30 June 1963. During this survey, five families, 15 genera, 31 species numbering 268 specimens were collected. The list of flies taken is shown in Table 12.

Of Of these flies, Lispe orientalis, Isomyia senomera, Sarcophaga fieldi, S. hokurikuensis, S. musashinensis, S. schutzei, S. septentrionalis and S. unguitigris were newly recorded from Shikoku.

Table 12. Medically Important Flies Collected in Kagawa and Ehime Prefectures, Shikoku District 24 - 30 June 1963

Family	Female F / Male M	Locality: Kotohira, Kagawa M	Kotohira, Kagawa F	Omogo-kei, Ehime M	Omogo-kei, Ehime F	Total M	Total F	Grand Total
Muscidae								
Graphomyia maculata		1			7	1	7	8
Lispe orientalis		3	3		2	3	5	8
Muscina angustifrons		4	4			4	4	8
Muscina stabulans		4	1	6		10	1	11
Orthellia latipalpis			2				2	2
Anthomyiidae								
Anthomyia illocata			2				2	2
Fannia canicularis		1	1			1	1	2
Ophyra leucostoma		2	7			2	7	9
Calliphoridae								
Aldrichina grahami		1	1		2	1	3	4
Chrysomya pinguis			2		1		3	3
Hemipyrellia ligurriens		1				1		1
Isomyia senomera					1		1	1
Lucilia ampullacea		4	6		1	4	7	11
Lucilia caesar		3	11		4	3	15	18
Lucilia illustris		1	2			1	2	3
Lucilia papuensis		2			2	2	2	4
Lucilia porphyrina		2	6			2	6	8
Phaenicia cuprina		1	1			1	1	2
Phaenicia sericata		3	2			3	2	5
Sarcophagidae								
Sarcophaga albiceps		3	2	5	1	8	3	11
Sarcophaga fieldi				1		1		1
Sarcophaga hokurikuensis				10	1	10	1	11
Sarcophaga melanura		8	6			8	6	14
Sarcophaga musashinensis				3		3		3
Sarcophaga peregrina		22	27	42		64	27	91
Sarcophaga schutzei					1		1	1
Sarcophaga septentrionalis				1		1		1

Table 12 (Cont'd)

Family	Kotohira, Kagawa M	Kotohira, Kagawa F	Omogo-kei, Ehime M	Omogo-kei, Ehime F	Total M	Total F	Grand Total
Sarcophaga similis	7	8			7	8	15
Sarcophaga tushimae	1				1		1
Sarcophaga unguitigris	1				1		1
Dryomyzidae							
Stenodryomyza formosa			7	2	7	2	9
	2				2		2
Total	77	94	75	25	152	119	271

Survey in Kyushu District: This survey was performed in Bungo-takeda and Mt. Suishi (Ogatacho), Oita Prefecture during 24 - 30 June 1963. The purpose of this survey was to collect fly specimens especially Sarcophaga oitana which had not been previously collected by the contractor. During this survey, 172 fly specimens were collected. These specimens consisted of four families, 10 genera and 29 species. Many rare species of sarcophagid flies such as Sarcophaga beesoni, S. hozawai, S. hakusana, S. konakovi and S. kinoshitai were collected. Moreover, a specimen of an extremely rare species of sarcophagid, Sarcophaga oitana, was obtained. Only three male specimens of this species were collected by Dr. Katsushige Hori on the summit of Mt. Suishi, Oita Prefecture. The list of flies collected is shown in Table 13.

Table 13. Medically Important Flies Collected in Oita Prefecture 24 - 30 June 1963

Family	Locality: Mt. Suishi Male	Mt. Suishi Female	Takeda Male	Takeda Female	Total Male	Total Female	Grand Total
Anthomyiidae							
Fannia scalaris	1				1		1
Muscidae							
Muscina angustifrons	15				15		15
Musca convexifrons	2				2		2
Stomoxys calcitrans		1				1	1
Calliphoridae							
Lucilia ampullacea		1				1	1
Lucilia papuensis		1				1	1
Chrysomya pinguis	1	1			1	1	2
Hemyipyrellia ligurriens				1		1	1
Melinda okazakii		1				1	1
Melinda tsukamotoi	1	1			1	1	2

Table 13 (Cont'd)

Family	Mt. Suishi Male	Mt. Suishi Female	Takeda Male	Takeda Female	Total Male	Total Female	Grand Total
Sarcophagidae							
Sarcophaga kinoshitai	1				1		1
Sarcophaga peregrina	3	4			3	4	7
Sarcophaga melanura	6				6		6
Sarcophaga albiceps	5	3			5	3	8
Sarcophaga similis	2	3		1	2	4	6
Sarcophaga tsushimae	19	2	5		24	2	26
Sarcophaga musashinensis			7		7		7
Sarcophaga fieldi	4		2		6		6
Sarcophaga konakovi	13				13		13
Sarcophaga hozawai	24				24		24
Sarcophaga kagaensis	11				11		11
Sarcophaga schutzei	14	1			14	1	15
Sarcophaga beesoni	2				2		2
Sarcophaga unguitigris	1				1		1
Sarcophaga shiritakaensis	1				1		1
Sarcophaga nakusana	6				6		6
Sarcophaga tuberosa	2				2		2
Sarcophaga oitana	1				1		1
Blaesoxypha japonensis	2	—	—	—	2	—	2
Total	137	19	14	2	143	29	172

Investigation of Muscoid Flies as Vectors of Thelaziasis: Thelaziasis is caused by a nematode eye parasite of cattle. This investigation was performed in cooperation with the Department of Medical Zoology, Tokyo Medical and Dental University and the Department of Hygiene, Niikappu Livestock Breeding Station, Ministry of Agriculture, from September 1962 - July 1963.

Members of the Niikappu Livestock Breeding Station who cooperated in this study are: Mr. Nobumasa Shimizu; Mr. Toshimasa Akamatsu; Mr. Asakichi Nagashima; Mr. Shoji Nagaoka; Mr. Rokuro Ebina; Mr. Katsuki Hagino and Mr. Kuniharu Morita.

The purpose of this investigation was to conduct an epidemiological study of Thelazia infestation in cattle; particularily, to determine the role of muscoid flies as vectors of this disease.

The seasonal occurrence of flies attracted to cows was observed in the Niikappu Pasture. Flies attracted to hitched cows were collected with an insect net for thirty minutes every month. Data obtained are shown in Table 14. Accordingly, it was found that four species of muscoid flies attracted to cattle, especially Musca convexifrons and Morellia simplicissima, gathered around the eyes of the animals. No flies were found on cattle between November and April. Females of M. convexifrons and M. simplicissima, outnumbered the males of these species. Peak populations of M. convexifrons were found in June.

Table 14. Seasonal Occurrence of Flies Attracted to Cows in Niikappu Pasture, Shizunai, Hokkaido
October 1962 - July 1963 *

	Date																			
	6 Oct		27 Oct		10 Nov		26 Nov		11 May		24 May		9 Jun		27 Jun		Total		Grand Total
Hitching Time	1310 - 1340		1400 - 1430		1300 - 1330		1310 1340		1300 - 1330		1300 - 1330		1000 - 1030		1330 - 1400				
Weather	Fine		Fine		Fine		Fine		Fine		Cloudy		Fine		Fine				
Temperature (H)	15 C		14 C		9.5 C		1 C		17 C		16.5 C		15.5 C		28 C				
Temperature (L)	-1 C		-1 C		-1.5 C		-6 C		1.5 C		0.5 C		2 C		13 C				
Wind velocity	2-3m		1-2m		1m		0-9.2m		1m		0		4m		2m				
Rain fall	0		0		0		0		0		27.1mm		0		0				
** Species	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Musca convexifrons	0	3	0	0	0	0	0	0	0	0	0	0	2	29	2	129	4	161	165
Morellia simplicissima	0	0	0	0	0	0	0	0	0	1	1	4	0	2	1	29	2	36	38
Stomoxys calcitrans	13	19	16	15	3	7	0	0	0	0	0	1	0	0	0	0	32	42	74
Lyperosia exigua	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	13	23	16	15	3	7	0	0	0	1	1	5	2	31	3	158	38	240	278

* During December 1962 - April 1963, no fly specimens were obtained.
** ♂ Male
♀ Female

Table 15 shows that 3971 muscoid flies were dissected during August 1962 - July 1963, and 165 thelazian larvae were obtained from 68 females of Musca convexifrons. The dissected flies consisted of 3,536 M. convexifrons (305 males and 3,231 females), 56 M. domestica vicina (32 males and 24 females), 318 Morellia simplicissima (2 males and 316 females), and 61 Stomoxys calcitrans (14 males and 47 females).

Thelazian larvae were found only in females of M. convexifrons. Therefore, it is assumed that only females of M. convexifrons can be vectors of thelaziasis in Niikappu. This was the first such finding in Japan. Numbers of thelazian larvae found in one fly varied from one to fifteen. These larvae were found predominantly in the abdomen of female flies rather than other organs or body regions such as proboscis, head or thorax. Eyes of 71 cows (5 or more each month) were examined. One hundred forty seven specimens of Thelazia rhodesi (40 males, 84 females and 23 larvae) were taken from eyes of 36 cows from August 1962 - July 1963 (Table 16).

Observation of Life Cycles of Some Fly Species in the Laboratory: The life cycles of 10 medically important fly species were observed in the insectary (regulated at 23°C ± 2°C). In these experiments, 300 ± 50 flies of each species were used. Duration in hours of each stage of development from egg to adult was observed. Life cycles of several medically important flies bred in the insectary is shown in Table 17. It was found that it usually took 12-16 days at 23°C ± 2°C for these flies to develop from eggs to adults. Artificial murine food was used as bait for breeding muscoid flies, and horse flesh was used for the calliphorid and sarcophagid flies.

Descriptions of New Species and Previously Unrecorded Species of Sarcophagid Flies: During this report period, descriptions of five new species were made. Of these, two of the new species were Sarcophaga asahinai and S. fieldi and the newly recorded species were S. pseudoscoparis. Reports on the description of these species will be published in professional journals at a later date. Descriptions of three other new species are now in press.

Observations on Distribution and Seasonal Occurrence of Medically Important Flies: This study was started in April 1963, in various prefectures throughout Japan. Results will be given in the next annual report.

List of Publications: KANO, R. 1962. Notes on flies of medical importance in Japan Part XVII. Description of two new species of genus Sarcophaga (Sarcophagidae, Diptera). Japanese J. Sanitary Zool. 13(4):235-239.

KANO, R. and PARK, Soung Ho. 1963. New records of Sarcophaga pseudoscoparia Kramer, 1911 in Japan and Korea. Japanese J. Sanitary Zool. 14(2):95-96.

Bionomics of Culex tritaeniorhynchus

The laboratory colony of Culex tritaeniorhynchus first established in 1956 is still maintained in the department. There have been no significant changes noted in behavior of colony specimens. During attempts to collect C. tritaeniorhynchus from overwintering habitats, behavior of adult C. pipiens, C. orientalis, C. hayashii and Anopheles sinsensis was observed in a cave near Atsugi Air Station. It was found

Table 15. Flies Infested by Thelazian Larvae in Niikappu Pasture
August 1962 - July 1963*

Male - M Female - F	August				September				October				June 1963			
	Dissected		Infested		Dissected		Infested		Dissected		Infested		Dissected		Infested	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Musca convexifrons	49	966	0	4	20	838	0	48	0	120	0	12	64	478	0	0
Musca domestica vicina	32	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Morellia simplicissima	1	67	0	0	1	72	0	0	0	0	0	0	0	0	0	0
Stomoxys calcitrans	14	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*During November 1962 - May 1963 no fly specimens were obtained.

Table 16. Thelazia rhodesi Found in Eyes of Cows
August 1962 - July 1963

	Month											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Number of cows examined	5	5	5	5	5	5	5	5	5	10	10	65
Number of infested cows	5	4	4	4	3	2	2	4	3	1	1	33
Positive ratio (Percentage)	100	80	80	80	60	40	40	80	60	10	10	58
Number of Thelazia rhodesi												
Male	12	6	3	4	7	1	0	3	2	0	0	38
Female	22	2	16	7	4	1	7	14	5	1	2	81
Larva	16	4	0	0	0	0	0	0	0	0	0	20
Total	50	12	19	11	11	2	7	17	7	1	2	139

Table 17. Life Cycles of Medically Important Flies Bred in Insectary (regulated at 23°C ± 2°C)

	Duration in Hours of Each Stage of Development					
	Egg	1st Instar	2nd Instar	3rd Instar	Pupa	Emergence oviposition
Musca domestica vicina (Tokyo)	24	24	36	96	108	196
Musca domestica vicina (Delhi)	12	36	36	120	96	168
Musca domestica vicina (YE)	24	24	24	84	132	
Musca domestica domestica (NAIDM)	24	24	24	72	132	
Fannia canicularis	24	48	24	180	164	
Phaenicia cuprina	12	24	24	156	168	
Phaenicia sericata	12	24	24	132	144	
Chrysomya pinguis	24	24	24	156	72	
Phormia regina	12	24	24	180	96	
Protophormia terraenovae	24	24	24	84	144	
Aldrichina grahami	24	24	24	132	180	
Sarcophaga crassipalpis		24	24	132	192	
Sarcophaga peregrina		24	24	144	192	

that, even at temperatures as low as 2-3°C, there was almost daily movement of specimens to new resting places in the cave. No overwintering C. tritaeniorhynchus was taken from caves later than November. Presence of engorged adult females in light trap collections on 16 March, and 18, 19, 25 and 29 April indicated that these specimens probably overwintered as adults, perhaps in some undiscovered habitat. The average female C. tritaeniorhynchus in the laboratory colony takes four blood meals and oviposits three times during a life span. Preliminary observations indicated that all females which consumed blood meals of 1.6 mg or over later oviposited. Many females which consumed blood meals of 1.5 mg or less did not oviposit. Ova developed in eight of ten females of C. tritaeniorhynchus fed on a 20 per cent solution of royal jelly and sugar water in lieu of a blood meal. Six of ten females fed on a ten per cent solution developed ova. One of these oviposited, the remainder were dissected.

Biochemical studies on adult C. tritaeniorhynchus and C. pipiens were continued. First, a biochemical analysis concerning amino acids, carbohydrates and fats was made. Next, a study on variation in lipid concentration of adult C. tritaeniorhynchus and C. pipiens maintained under temperatures encountered in the Tokyo area during summer and winter months was conducted. An autogenous strain of Culex pipiens molestus, established from specimens collected near Tokyo, which is now in the third generation continues to be maintained in the laboratory. An attempt is being made to colonize Anopheles sinensis utilizing specimens collected in the Camp Zama area.

A Comparative Study of the Effect of Temperature on Lipid Concentration in Two Species of Adult Mosquitoes: Results of biochemical analyses of amino acid, carbohydrate and fat content of adult mosquitoes were reported previously in the Annual Professional Report, January 1961 - June 1962, Medical General Laboratory (406). This report covers variation in lipid concentration caused by change in temperature under which adult C. tritaeniorhynchus and C. pipiens were maintained. The report is divided into the following sections:

1. Determination of Lipid Contents.
2. Analysis of Lipid Components
3. Analysis of Glycerides.
4. Analysis of Fatty Acids.

The C. pipiens which were used in this determination originated from egg rafts collected in Kanagawa Prefecture; and C. tritaeniorhynchus material was obtained from a strain maintained in the laboratory. Throughout the study these two species were reared under similar conditions of larval concentration, temperature and availability of food. Two-day old adults of both species were maintained in cages which were kept at 25°C, 15°C and 4°C respectively. During the experiments they were fed on a 15 per cent sugar solution.

At 48 hour intervals 100 adults from each group were removed from their cages and killed by placing them in a deep freeze held at -10°C. Their dry weight was determined after they had been placed in a drying chamber for three hours at 70-80°C. The simple lipids were extracted with petroleum ether (b.p. 40-60°C) in a Soxhlet's apparatus for 10 hours. Following the extraction they were dried and reweighed to determine the weight of extracted simple lipids. The conjugated lipids were extracted with absolute ethanol in a Soxhlet's apparatus for 10 hours. Again the mosquitoes were dried and weighed to determine the amount of conjugated lipids which had been extracted. The difference in weight before and after the petroleum ether extraction was taken as the weight of the simple lipids, and the difference in weight before and after the ethanol extraction was taken as the conjugated lipids. The difference in weight between the original dry weight and the weight after the ethanol extraction represented the amount of total lipids.

Figures 1 - 10 show simple lipids, conjugated lipids and total lipid content percentage for each dry weight of C. tritaeniorhynchus and C. pipiens adults held at each rearing temperature.

Figures 1 - 3 show that the lipid content of C. tritaeniorhynchus increases progressively throughout a six day period, and lipids are exhausted in two days after which the lipid content increases progressively for the following six days. These results indicate that one period of lipid metabolism in C. tritaeniorhynchus

Lipid Levels in C. tritaeniorhynchus Females Held at 25° C

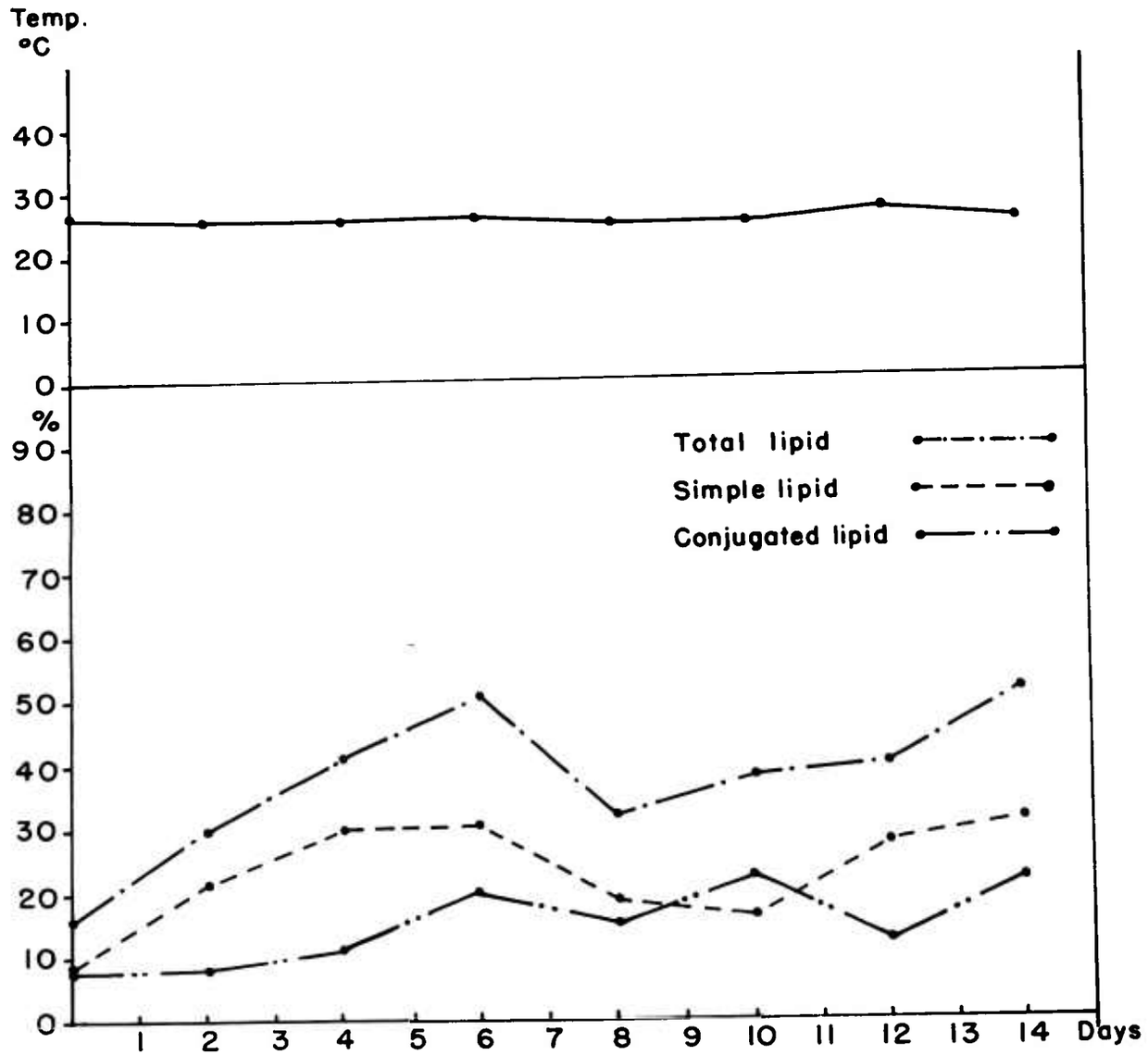


Figure 1.

Lipid Levels in C. tritaeniorhynchus Females Held at 15° C

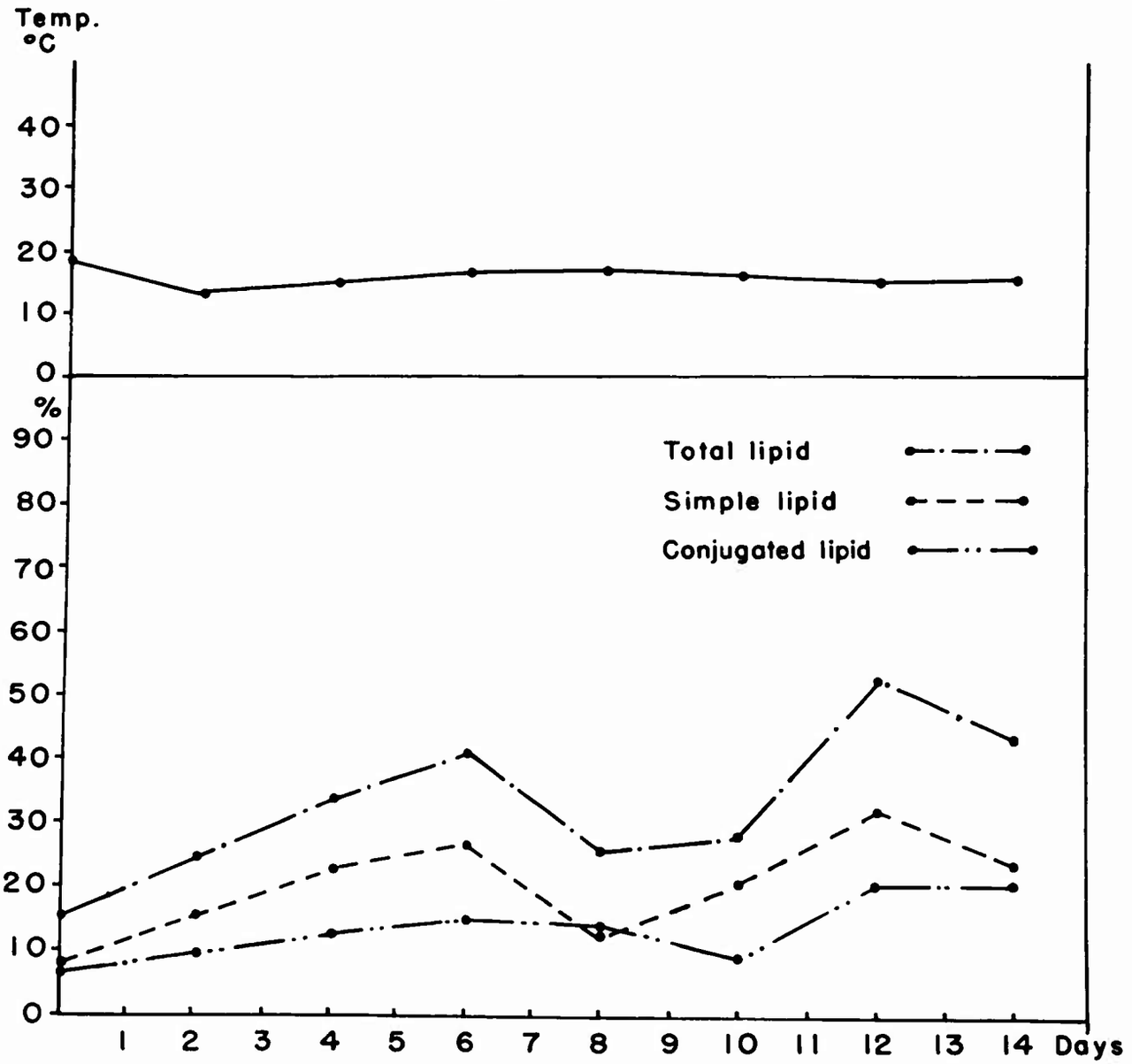
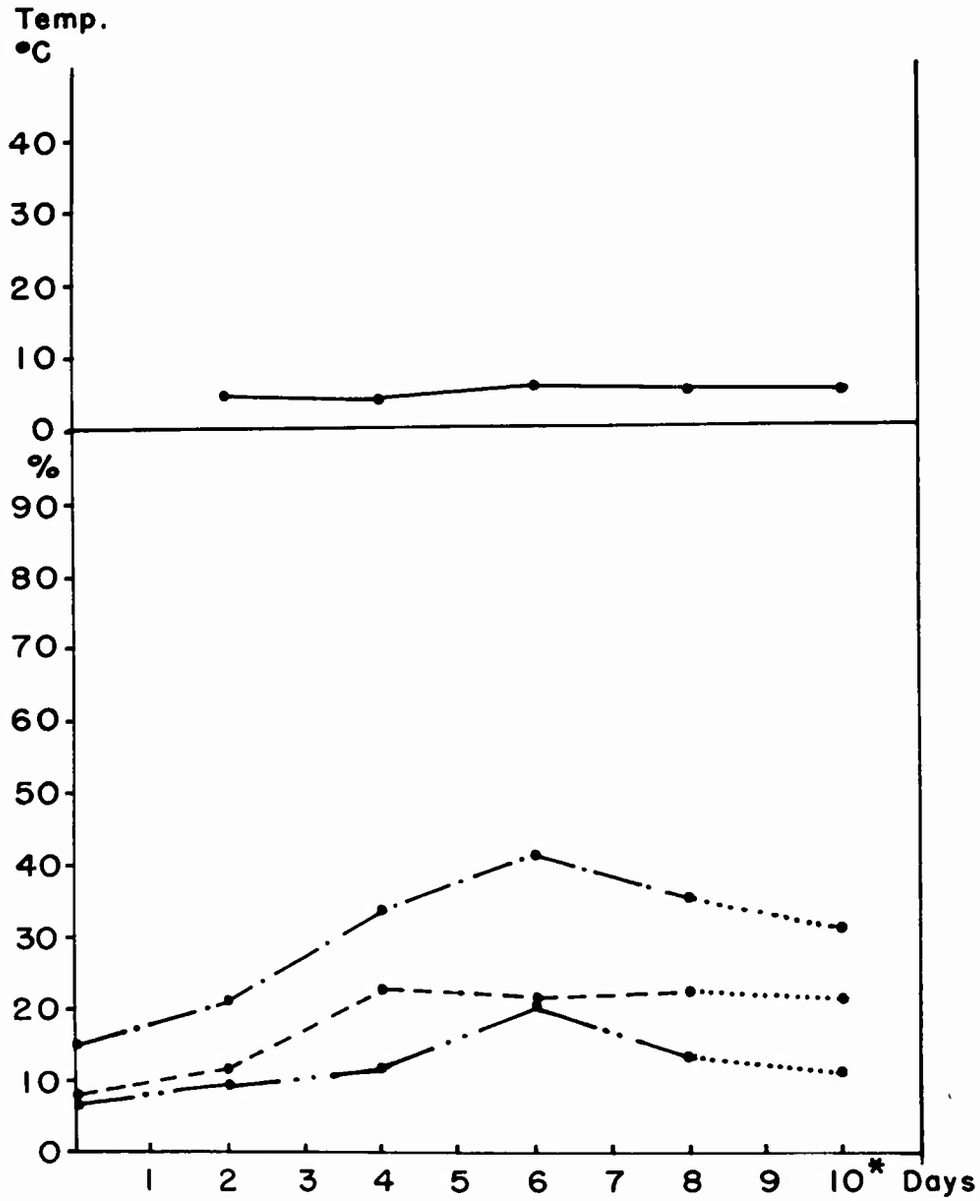


Figure 2.

Lipid Levels in C. tritaeniorhynchus Females Held at 4°C



* Determinations made on Mosquitoes which died on the 10th day.

Figure 3.

is stable for six days when the mosquitoes are given adequate food and held at high temperature. Specimens of C. tritaeniorhynchus were dead on the 10th day at 4°C; however, this was not due to lipid expenditure. Further studies on this lipid metabolism using C^{14} glucose should yield interesting results.

Figure 4 indicates that the simple lipids increased at 15°C, but that conjugated lipids decreased as respiration decreased. This may be due either to effects of feeding or to change in simple lipids. Therefore, further studies on the respiratory quotient (RQ) and on the carbohydrates are needed. It is believed that simple lipids and conjugated lipids decreased at 4°C, as shown in Figure 5, because C. tritaeniorhynchus did not feed at this temperature.

The course of lipids in C. pipiens is different than in C. tritaeniorhynchus. The course of the simple lipids and the conjugated lipids were inverse, and period of lipid metabolism could not be found in C. pipiens. The simple lipids, however, were present in much greater amounts than the conjugated lipids upon variation of temperature. These results are shown in Figure 7. The simple lipids formed a greater percentage of the total than the conjugated lipids as shown in Figure 8 and 9. Lack of reduction of total lipids (shown in Figure 10), may indicate that the total lipid count was not decreased, therefore it can be postulated that C. pipiens were able to feed at 4°C.

This action of lipids may indicate that C. pipiens and C. tritaeniorhynchus overwinter in different habitats.

The lipid contents of adult C. pipiens collected in Kanagawa Prefecture during November 1962 - February 1963, C. tritaeniorhynchus and C. pipiens reared at 25°C, 15°C and 4°C are shown in Table 18. The percentage of simple lipids was greater than that of conjugated lipids in wild-caught C. pipiens. This indicates that specimens of C. pipiens, collected in the field, overwinter in similar conditions as shown in Figures 9 and 10.

Analysis of Lipid Components: Paper chromatographic analysis of simple lipids and conjugated lipids were carried out. Twenty mosquitoes, which had been held four days at 15°C and fed on a 15 per cent sugar solution, were used as the material for samples two and five. The mosquitoes were extracted with ethanol in a tissue grinder. Samples three, four, six and seven were used for petroleum ether extraction and for ethanol extraction as described in this report.

A sodium silicate solution was employed for paper impregnation. Whatman No. 1 filter paper was impregnated by being passed through diluted silicate solution, suspending it for approximately five minutes, and immersing it in 6NHcl for 30 minutes. This paper was then washed with running tap water, distilled water and suspended to dry. Chromatograms were carried out at 25°C by the ascending migration method. The mobile solvent was Di-isobutyl ketone-acetic acid-water (40:30:7). The detecting agent was composed of 0.02 per cent Rhodamine B solution, 0.2 per cent ninhydrine-butanol solution, and phosphomolybdic acid reagent.

Results of these lipid analyses by paper chromatography are described in Figure 11. The same types of lipid were present in C. tritaeniorhynchus and C. pipiens. The simple lipids were composed of mono-, di, tri-glyceride and fatty acid but the conjugated lipids included inositolphospholipid, phosphatidylserine, lecithine, chloresterin and non-phospholipid.

Lipid Levels in C. tritaeniorhynchus Females
Held at Varying Temperatures (1)

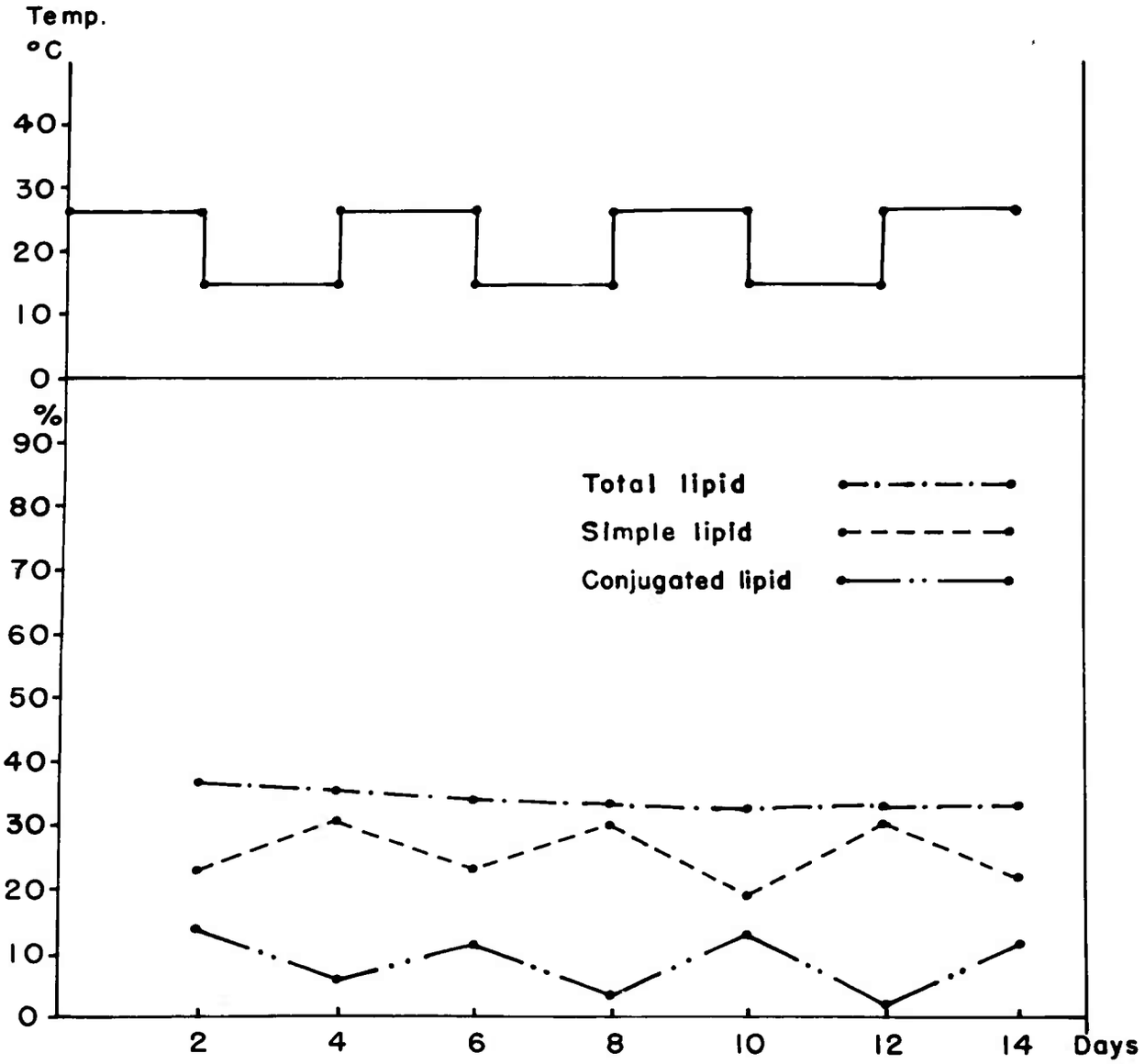


Figure 4.

Lipid Levels in C. tritaeniorhynchus Females Held at Varying Temperatures (2)

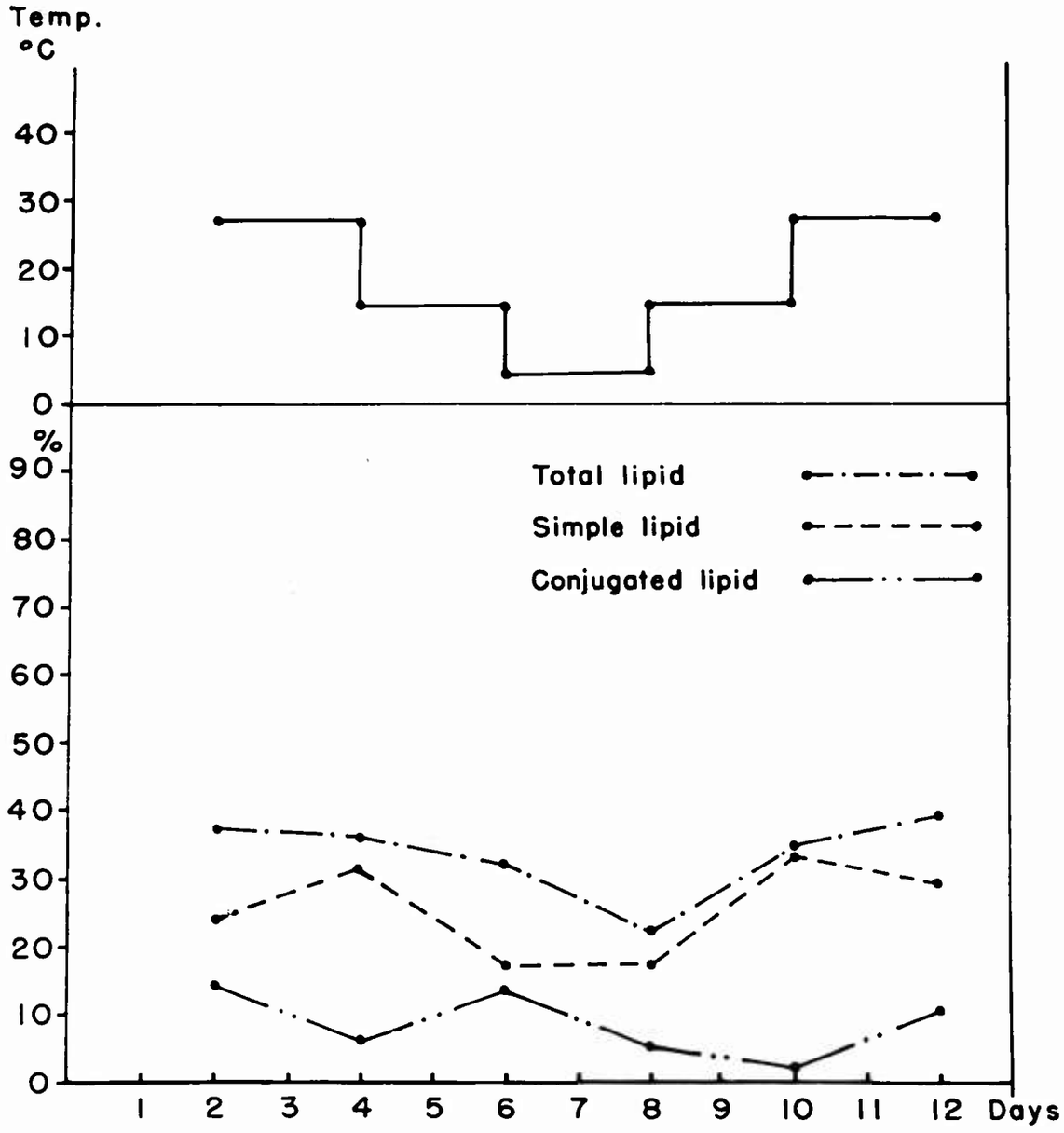


Figure 5.

Lipid Levels in C. pipiens Females Held at 25° C

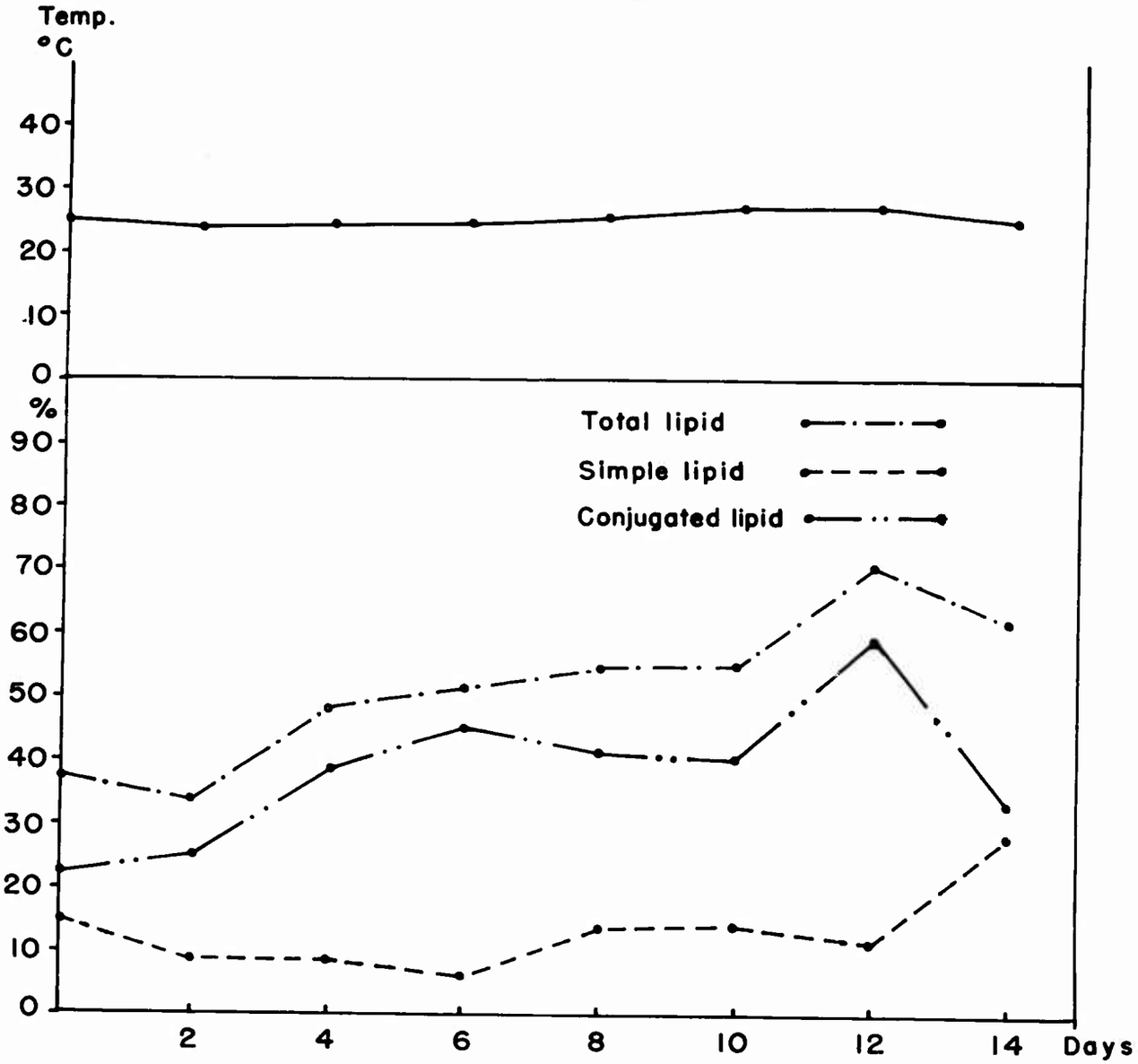


Figure 6.

Lipid Levels in C. pipiens Females Held at 15°C

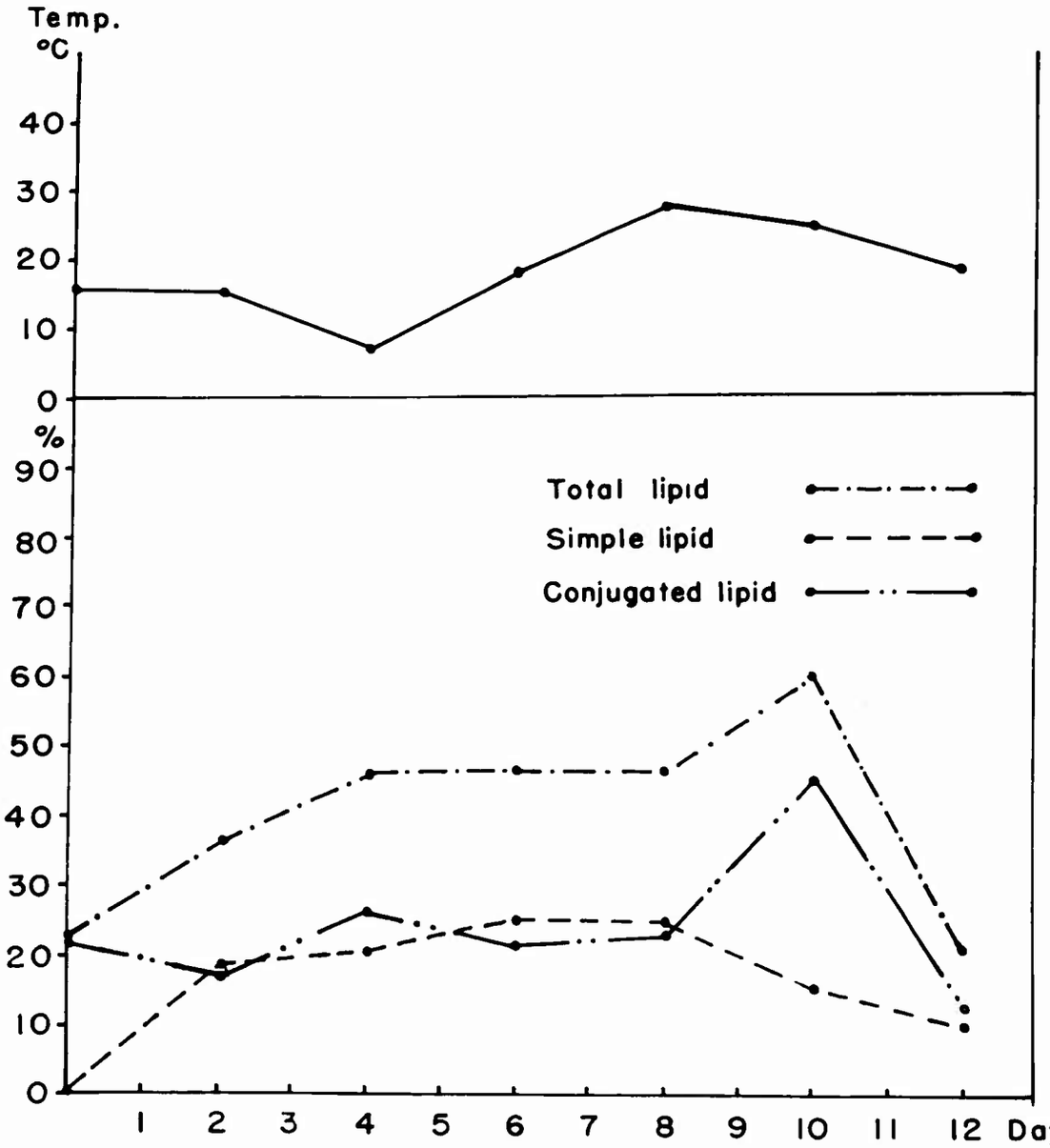


Figure 7.

Lipid Levels in C. pipiens Females Held at 4° C

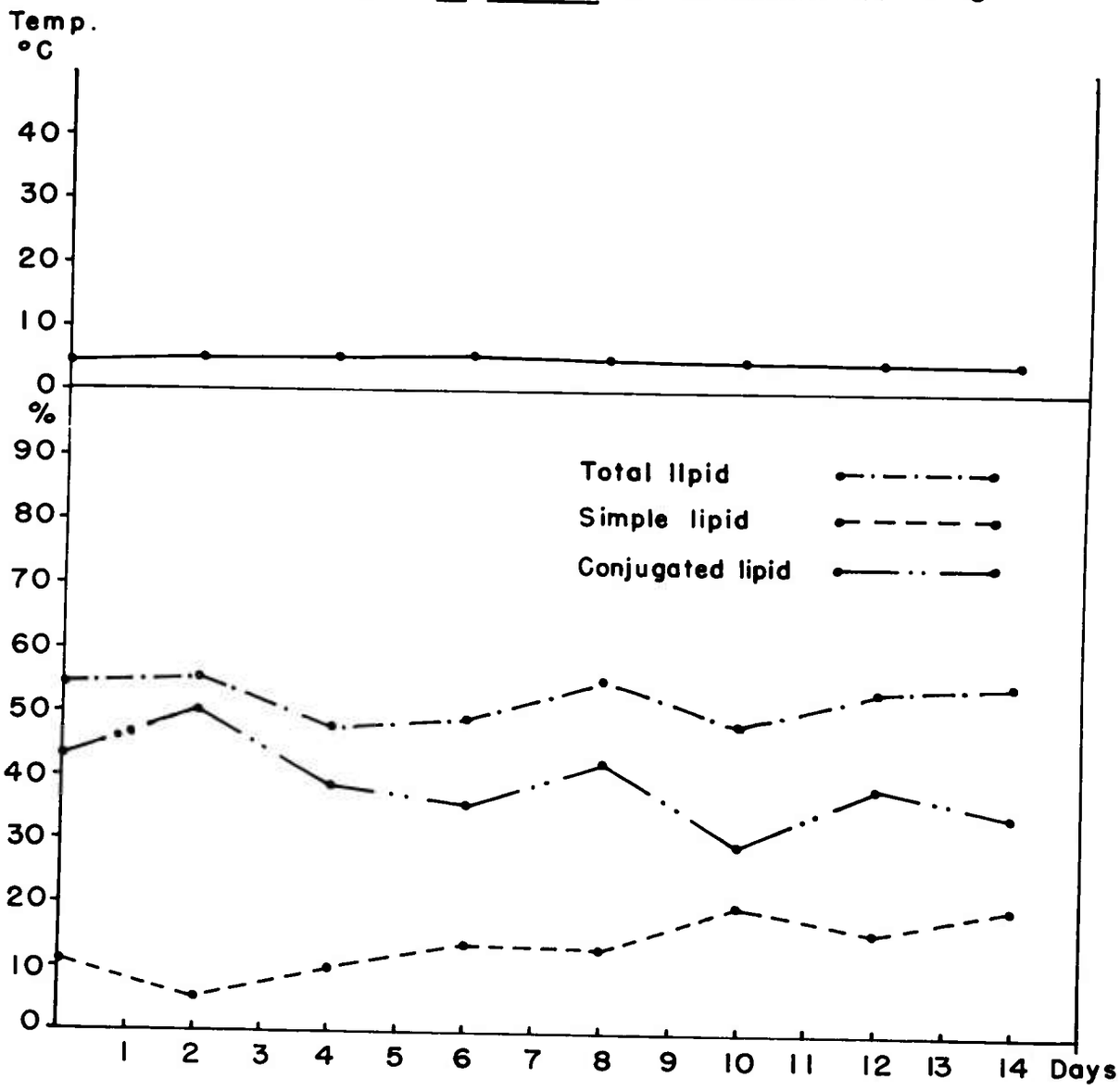


Figure 8.

Lipid Levels in C. pipiens Females Held at Varying Temperatures (1)

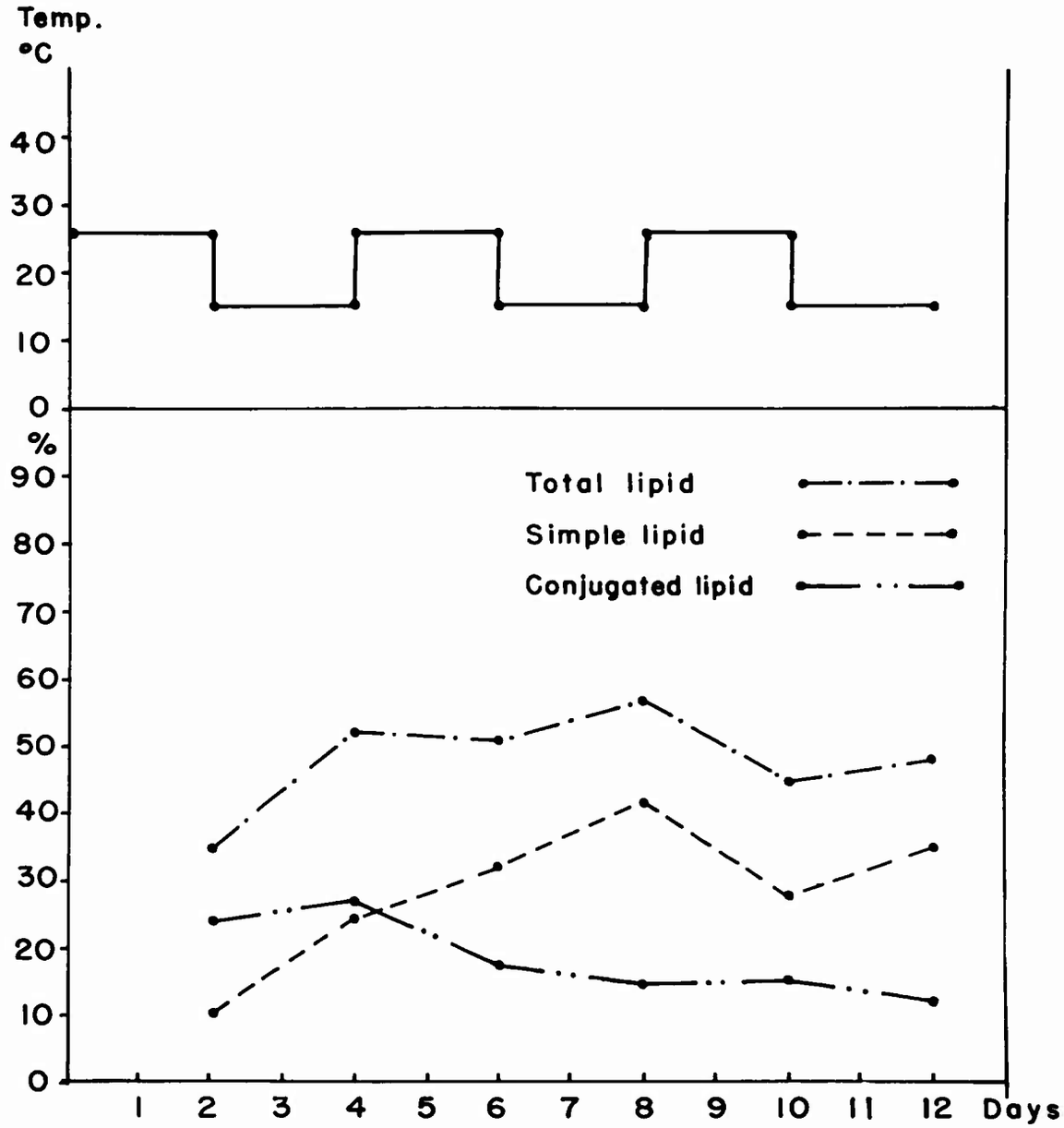


Figure 9.

Lipid Levels in C. pipiens Females Held at Varying Temperatures (2)

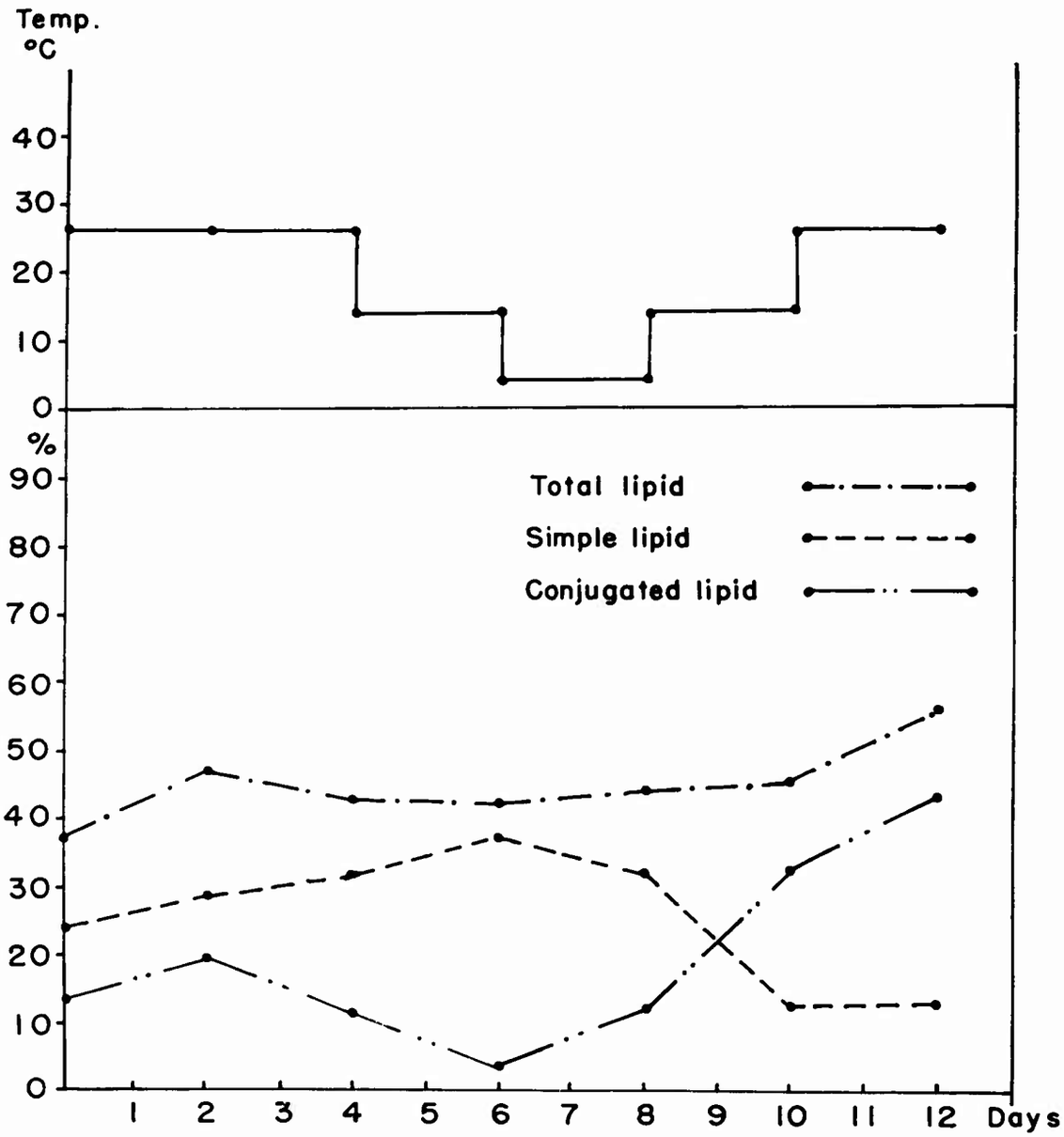
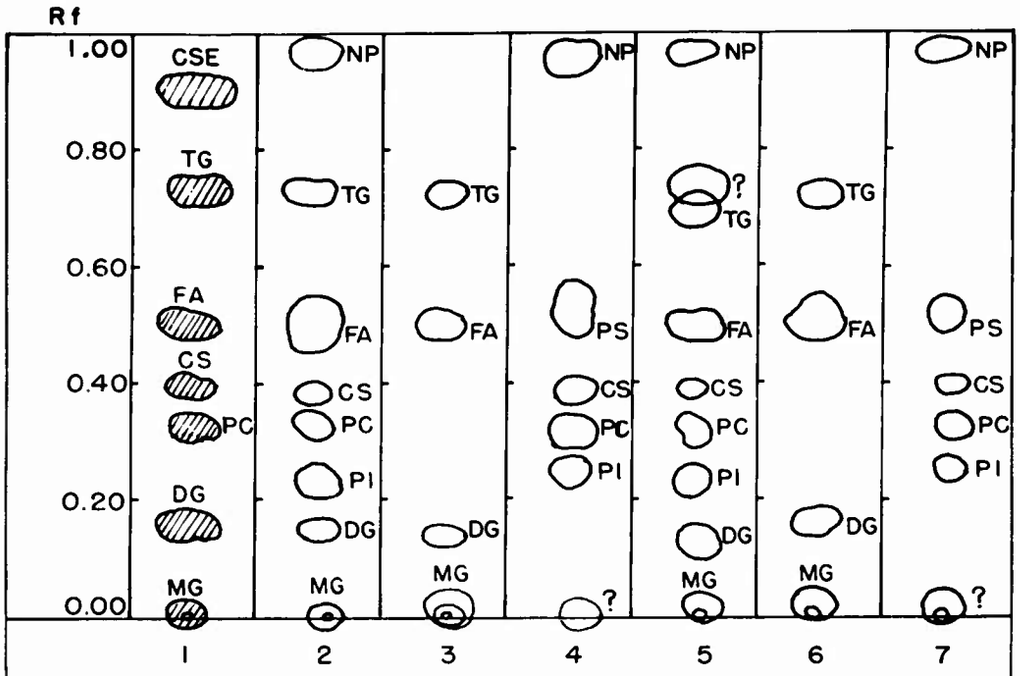


Figure 10.

Paper Chromatograms of Lipids in Mosquito Tissue



1. Rf Value of Reference
2. homogenate of C. tritaeniorhynchus with ethanol
3. petroleum ether extraction of C. tritaeniorhynchus
4. ethanol extraction of C. tritaeniorhynchus
5. homogenate C. pipiens with ethanol
6. petroleum ether extraction of C. pipiens
7. ethanol extraction of C. pipiens

MG; Monoglyceride
DG; Diglyceride
PC; Lesitine
CS; Cholesterol
FA; Fatty acid
TG; Triglyceride
CSE; Cholesterol ester
PI; Inositol-phosphalipids
PS; Phosphatidylserine
NP; Non-phospholipids

Figure 11.

Table 18. Dry Matter Weight and Lipid Content of Reared Mosquitoes and Adults Collected During Hibernation

	Rearing Temperature	Dry Matter Weight(mg)	Simple Lipid		Conjugated Lipid		Total Lipid Per Cent
			Weight(mg)	Per Cent	Weight(mg)	Per Cent	
C. tritaeniorhynchus	25°C	88.1	26.0	29.5	9.6	10.9	40.4
	15°C	80.6	18.0	22.3	9.8	12.1	34.4
	4°C	74.6	17.0	22.7	8.6	11.5	34.2
C. pipiens	25°C	94.8	8.4	8.9	37.6	39.7	48.6
	15°C	61.6	12.4	20.1	16.0	26.0	46.1
	4°C	104.0	10.2	9.8	40.2	38.7	48.5
C. pipiens (collected)							
20 Nov		136.6	56.6	41.4	13.0	10.5	51.9
4 Dec		124.5	49.6	39.8	9.7	12.8	52.6
25 Dec		117.9	43.9	36.4	8.6	13.7	50.1
8 Jan		124.7	38.3	30.7	10.4	12.0	42.9
21 Jan		197.1	69.0	35.0	12.6	15.5	50.6

Little is known about the biological role or transformation of the conjugated lipids in mosquitoes. Columnar chromatographic quantitative analysis of the variation of concentration caused by change in temperature should yield important results. On the other hand the role of polyhydric alcohol, which seems to be correlated with lipid metabolism and energy production, should be analyzed.

Analysis of Glycerides: Paper chromatographic analyses of glycerides in fat bodies and all lipids were conducted. See Figures 12 and 13. Sample two consisted of fat bodies floated out of 20 dissected female C. tritaeniorhynchus and C. pipiens which had been held at 13°C and fed on 15 per cent sugar solution for two days. Small quantities of a 0.9 per cent saline solution were used to float out the fat globules under a dissecting microscope. The saline solution with the fat globules was taken up on filter paper and then extracted with ether. Sample three consisted of tissue, without fat globules, of 20 dissected females. Samples one and four were composed of 20 males and 20 females respectively. After extraction with ether in a tissue grinder, each sample was then treated with excess mercuric acetate in 0.5 ml of methanol to which one drop of acetic acid was added. This was then heated at 80°C for 30 minutes, after which one ml of benzene and 10 ml of distilled water were added and the mixture thoroughly shaken.
The stationary solvent was 0.3 ml tetralin for Whatman No. 1 filter paper, 3 x 40 cm, and the mobile solvent was methanol-acetic acid (5:1). The detecting agent was 0.2 per cent diphenyl-carbazone-ethanol solution. The measurement of optical density (absorbance) of spots was accomplished with a densitometer with a yellow filter.

Results of the glyceride analysis by paper chromatography are shown in Figures 12 and 13. Spots A, B, and C were 1-parmit-3-oleic glyceride, 1,3-dioleic glyceride and trilinoleic glyceride respectively. It appears that the fat body contains many quantities of 1-3 dioleic glyceride and small quantities of trilinoleic glyceride is not found in the lipid of tissue without fat bodies. It is presumed that the reserve fat of fat bodies is usually in the form of 1,3-dioleic glyceride.

Analysis of Fatty Acids: The materials used were described earlier. Samples three and five consisted of hydrolized fat of 20 homogenized mosquitoes with 2 ml ethanol. A solution of 0.05N-KOH and ethanol was used to hydrolize the fat which was heated with a reflux condenser of a water bath for one hour. The other 20 mosquitoes were ground in 2 ml ethanol as samples two and four. Each was then neutralized with 0.05N-KOH ethanol solution with phenolphthalein as indicator. Each sample with 0.5 ml 1 per cent p-bromophenacyl-bromide was heated at 80°C for two hours with a reflux condenser. This was followed by the addition of 0.15 ml 1 per cent 2,4-dinitro phenyl hydrazine in 2NHCl-methanol solution. The material was then held for three hours at room temperature. Ether was then added to the reacting mixture. The ether extract was condensed to 1 ml after washing with distilled water five times. Petroleum hydrocarbon (b.p. 140-170°C) was used as the stationary solvent. The developing solvent used against the former was methanol-acetic acid-petroleum hydrocarbon (b.p. 140-170°C) (30:1:7).

As shown in Figure 14 it was found that three kinds of free fatty acids linolenic acid, linoleic acid and oleic acid were found in the tissues of C. tritaeniorhynchus and C. pipiens. However, the hydrolized tissue of those mosquitoes

Chromatograms of Glyceride in C. pipiens

(Optical density - Rf curve)

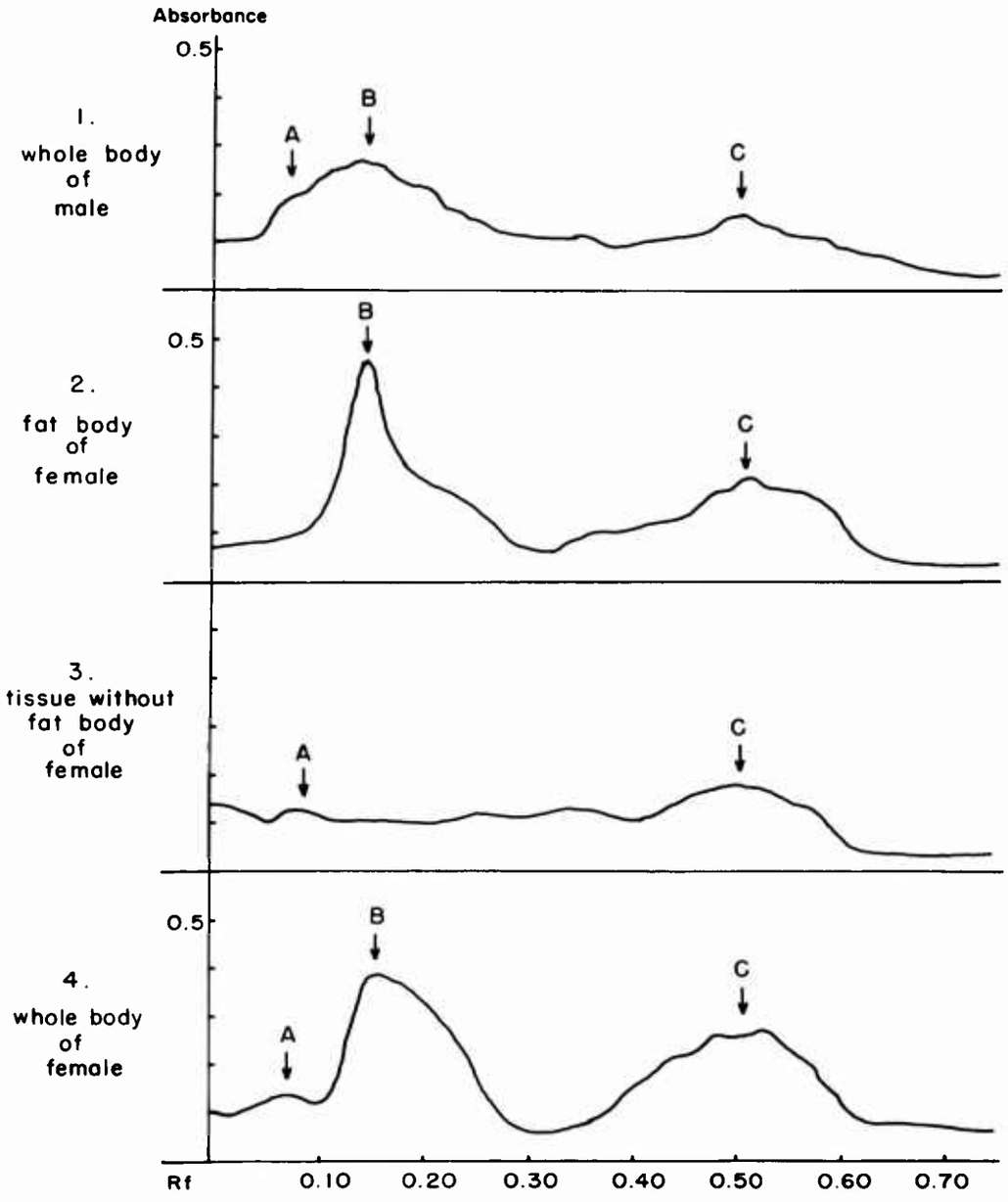


Figure 12.

Chromatograms of Glyceride in C. tritaeniorhynchus

(Absorbance - Rf curve)

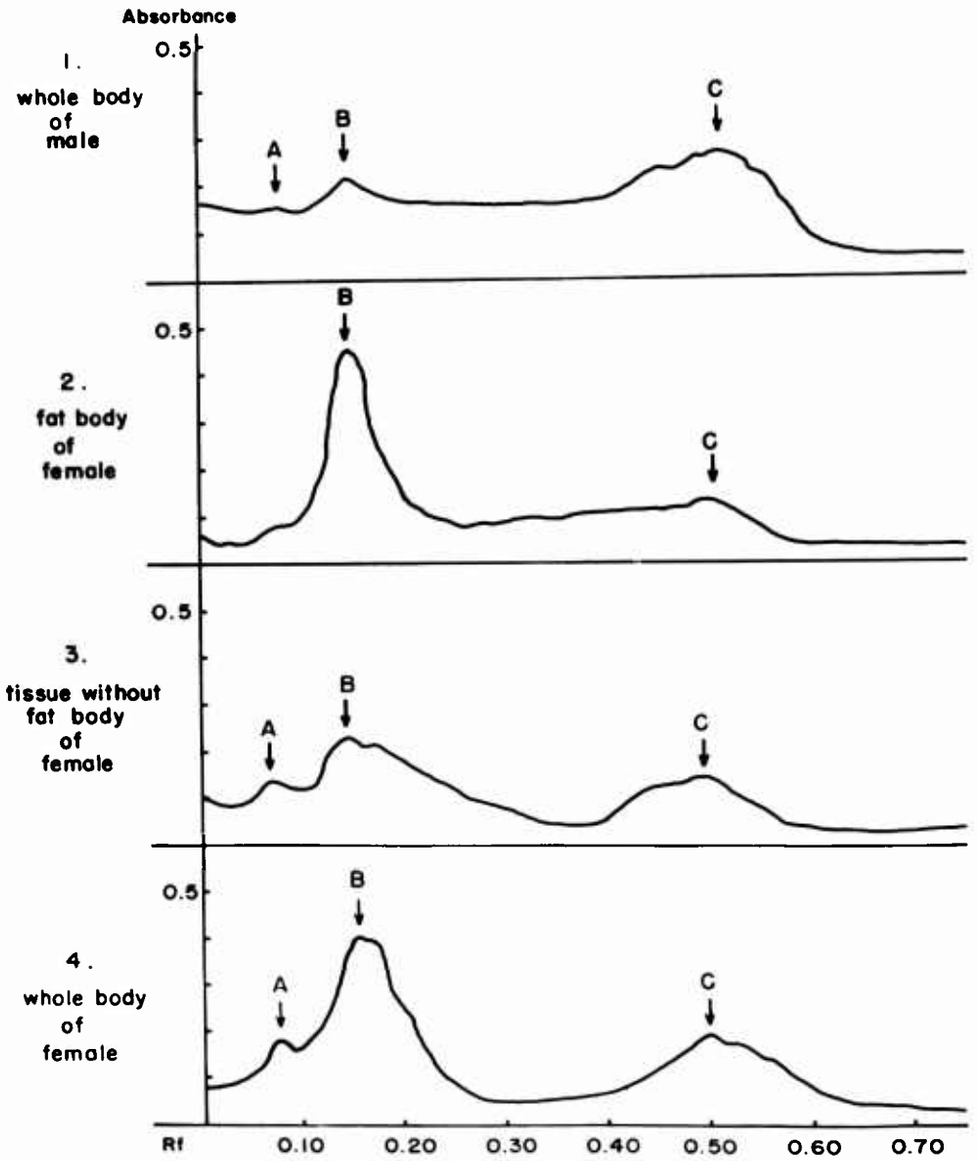


Figure 13

Paper Chromatograms of Fatty Acid Bromazine Ester in C. tritaeniorhynchus and C. pipiens

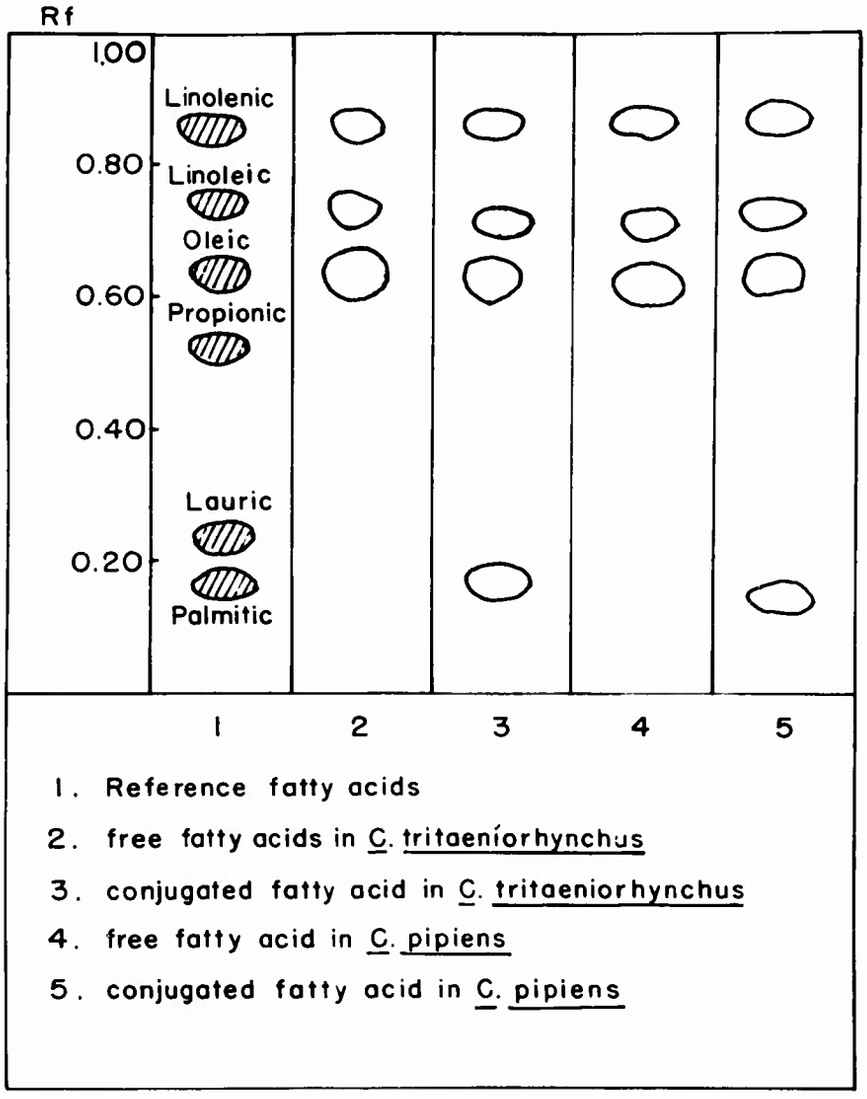


Figure 14.

contained four kinds of fatty acids - linolenic acid, linoleic acid, oleic acid and palmitic acid. The results are consistent with the results of the analyses of glyceride, described in this report earlier. Almost all of the parmitic acid was the component of 1-parmit-3-oleic glyceride in the body.

Quantitative analysis of these fatty acids of gaschromatography at varying temperatures is needed.

Field Collections

Although mosquito collections at U. S. Army Installations in Japan had been previously carried out in a fairly satisfactory manner, full cooperation of unit and installation commanders was obtained to insure proper surveillance of mosquito populations on and adjacent to USARJ installations. Publication of USARJ Circular No. 40-1, dated 14 March 1963, established the policies and procedures of the mosquito collection program. Provisions of this circular are as follows:

Mosquito collection and survey program. From 1 May to 1 October 1963, personnel of the U. S. Army Medical Command, Japan, will carry out an intensive mosquito collection and survey program in Japan. The primary objective of this program will be surveillance of the local populations of the Japanese encephalitis vector, C. tritaeniorhynchus, on and near military installations. The program will include tests to determine resistance of mosquitoes to insecticides and collection of adult and larval mosquitoes. Information obtained may indicate whether existing control measures are effective or whether different insecticides or control techniques should be applied for control of species that may transmit disease to man.

Assistance. While insecticide resistance tests, identification of specimens, and supplemental collections will be carried out by personnel of U. S. Army Medical Command, Japan, assistance in routine collections at each installation will include: 1) Operation of mosquito light traps three times weekly and 2) Collection of mosquito adults and larvae from designated stations once weekly.

Commanders will make personnel of the organizations available to assist in this program. It is recommended that personnel of installation medical facilities or of unit vector control details be identified for this purpose. During April 1963, personnel of the Medical General Laboratory (406), U. S. Army Medical Command, Japan, will visit each installation to instruct personnel assigned to the mosquito collection program in methods for collection, packing, and shipment of specimens to this laboratory for identification. Mosquito light traps and collecting material will be furnished by the Medical General Laboratory (406).

Species and numbers of adult and larval mosquitoes collected from 1 July 1962 - 30 June 1963 are shown in Tables 19 and 20.

Ecology and Control of Disease Vectors and Reservoirs

Studies on the Bionomics, Distribution and Control of Medically Important Scorpions: During FY 1963 progress on this project included preparation of illustrations of 14 species from various areas, observation on growth and behavior of specimens in laboratory colonies, and initiation of studies on spermatogenesis in

Table 19. Collections of Adult Mosquitoes on and near Camp Zama, Japan
1 July 1962 - 30 June 1963 *

Species	Sex	Collections Per Month: Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Aedes albopictus	M		4								161	32	7	204
	F	1	6								100	175	350	632
Aedes flavopictus	M												5	5
	F												143	143
Aedes japonicus	M										30			30
	F									40	82	5	11	138
Aedes koreicus	M													0
	F									1				1
Aedes nipponicus	M													0
	F												1	1
Aedes sinensis	M													0
	F			18										18
Aedes togoi	M			5							2			7
	F			60							8			68
Aedes vexans nipponii	M		1109	13							183	105	94	1,504
	F	2281	2500	407	32						468	823	313	6,824
Anopheles lindesayi japonicus	M													0
	F									4				4
Anopheles sinensis	M		169	4								1	3	177
	F	332	1255	152	[illegible]			1	1	[illegible]		4	14	1,770
Anopheles sineroides	M													0
	F							1	1	[illegible]				4
Armigeres hyrcanus	M		22	2										24
	F	252	334		5									591
Armigeres subalbatus	M		9	3	6							11	11	40
	F	8	19	29	11							71	114	252
Culex bitaeniorhynchus	M													0
	F	2	2	1	10									15
Culex hayashii	M							102	29	8	2	2	28	171
	F					335	3	411	573	432	217	121	54	2,151
Culex orientalis	M				1									1
	F	1				100	10	65	12	29	7		11	235
Culex pallidothorax	M													0
	F					2		4	1	2				9
Culex pipiens	M	14	125	34	2	23	2	27		1	524	450	294	1,496
	F	243	130	175	85	582	80	1711	1028	353	1287	6359	85287	97,321
Culex rubensis	M													0
	F												7	7
Culex ryukyensis	M													0
	F												17	17
Culex sasi	M										1			1
	F										4			4
Culex sinensis	M													0
	F		14											14
Culex tritaeniorhynchus	M		181		2									183
	F	3226	3701	495	50						19		4	7,495
Culex vorax	M		4											4
	F		2											2
Culiseta kanayamaensis	M													0
	F												18	18
Tripteroides bambusa	M													0
	F												5	5
Total	M	14	1623	61	11	23	2	129	29	9	903	601	447	3,852
	F	6346	7963	1337	200	1019	93	2193	1621	865	2195	7558	86344	117,734

* Specimens collected during November 1962 - March 1963 were taken by hand in overwintering habitats. All others were taken in light traps.

Table 20. Larval Collections
April - June 1963

Species	Collections Per Month				Total
	March	April	May	June	
Aedes albopictus		171	178	469	818
Aedes flavopictus				137	137
Aedes japonicus	40	37		37	114
Aedes togoi		10			10
Aedes vexans	17	480	50		547
Anopheles lindesayi	3				3
Anopheles sinensis				7	7
Anopheles sineroides				16	16
Armigeres subalbatus				85	85
Culex hayashii			120	11	131
Culex orientalis				26	26
Culex pipiens	12	1,705	5,720	131,820	140,257
Culex rubensis				7	7
Culex ryukyuensis				17	17
Culex sasai	3				3
Culex tritaeniorhynchus			5	1	6
Tripteroides bambusa				4	4
Total	75	2,403	6,073	132,637	141,188

species from India and Mexico. A paper describing techniques developed for maintenance of laboratory colonies of scorpions was published in the Bulletin of the World Health Organization, and an additional paper concerning medically important scorpions of the Pacific region was published in a symposium volume of the Tenth Pacific Science Congress.

Detailed observations were made on the growth of the Indian Buthotus tamulus scorpions. In a series of 15 gravid females of this species numbers of embroyos varied from 30-61. The mean was 41. During a six-month period young

scorpions in the colony were observed every day, and records were maintained of shedding and growth. One specimen shed on the third day following birth, and subsequently on the 36th, 56th, 91st, 141st and 193d days. Although the young scorpions fed well on small roaches, growth was extremely slow. Average length on the first day following birth was 10.3 mm. It was 13.64 mm on the fourth day, 14.2 mm on the sixth day, 15.8 mm at 30 days, 19.0 mm at 61 days, and 23.0 mm at 92 days. Throughout this period relative length of the post-abdomen shows graduate increases. Adult females of B. tamulus average 70 mm in length.

Young of Centruroides limpidus tecomanus and Tityus bahiensis showed equally slow growth rates. All specimens were maintained under the same conditions. Species present in the laboratory colony during the year included: Leiurus quinquestriatus (from Israel), Centruroides limpidus limpidus, C. limpidus tecomanus, C. elegans, C. suffussus (from Mexico), Centruroides vittatus (from Texas, U.S.A.), Tityus bahiensis and T. serrulatus (from Brazil), Heterometrus gravimanus and Buthotus tamulus (from India), and Androctonus bicolor (from Israel). Of the species kept in large numbers, only Heterometrus gravimanus has failed to produce young.

Studies on spermatogenesis in Heterometrus gravimanus, Buthotus tamulus, and Centruroides limpidus limpidus are now in early stages.

Development of a Polyvalent Antivenin for Treatment of Stings by all Known Species of Dangerously Venomous Scorpions: During FY 63 efforts were continued to find a suitable venom formula for production of a polyvalent antivenin which would be useful in treatment of scorpion sting throughout the world. In addition, available commercially produced scorpion antivenins were screened for neutralizing ability, and various mixtures of these were tested for effectiveness in neutralization of scorpion venoms from Mexico, South America, Turkey, Israel, India and Egypt. Because of the relatively low potency of most commercially prepared scorpion antivenins, all such mixtures were of rather limited effectiveness. For example, only undiluted homologous antivenin (in a volume of 0.50 ml) protected white mice against effects of intraperitoneal injection of three LD_{50}'s of Leiurus quinquestriatus venom taken from specimens collected in Israel. None of the available antivenins was as effective as might be desired against venom of the common Indian scorpion, Buthotus tamulus. An immunization program involving simultaneous injection of several venoms, and separate injection of individual venoms into rabbits and sheep has been underway since December 1962. Sera from these animals will not be assayed for potency until October or November 1963. Completion of the task may be delayed unless a new source for venom of the Turkish scorpion, Androctonus crassicauda, is discovered. Antivenin prepared with venom of this scorpion will neutralize venoms of some distantly related species. The reverse is not true.

Studies of Commerically Produced Antivenins: Screening tests with commercially produced snake antivenins were conducted during February, March and April 1963. Neutralization tests were discontinued after April due to lack of materials and priority of other departmental work, but will be resumed in October. During May and June additional supplies of antivenins from Taiwan, Thailand, India, Australia and South Africa arrived at the laboratory. The recently developed sea snake antivenin produced by the Commonwealth Serum Laboratories in cooperation with the Snake

and Venom Research Institute, Penang, Malaya, was among products received. This antivenin was prepared with venom of the widely distributed sea snake, Enhydrina schistosa, a major cause of sea snake bite in Southeast Asia. Its effectiveness against venoms of other species of sea snakes is not known, and an effort will be made to collect venoms of additional species for testing purposes. Table 21. gives information concerning antivenins now on hand for testing purposes.

Table 21. Antivenins Available in the Laboratory for Neutralization Tests Against Venoms of Southeast Asian Snakes

Antivenins	Country	Producer
Mamushi (monovalent)	Japan	Institute for Infectious Diseases, Tokyo
Habu (monovalent)	Japan	Institute for Infectious Diseases, Tokyo
Polyvalent hemorrhagic	Taiwan	Serum Vaccine Laboratory, Shiling, Taipei
Polyvalent neurotoxic	Taiwan	Serum Vaccine Laboratory, Shiling, Taipei
Taiwan cobra (monovalent)	Taiwan	Serum Vaccine Laboratory, Shiling, Taipei
Krait (monovalent)	Taiwan	Serum Vaccine Laboratory, Shiling, Taipei
Hundred-pace (monovalent)	Taiwan	Serum Vaccine Laboratory, Shiling, Taipei
Philippine corbra (monovalent)	Philippines	Serum Vaccine Laboratory, Alabang, Rizal, Luzon
Common cobra (monovalent)	Thailand	Queen Saovabha Institute, Bangkok
King cobra (monovalent)	Thailand	Queen Saovabha Institute, Bangkok
Krait (monovalent)	Thailand	Queen Saovabha Institute, Bangkok
Malayan pit-viper (Agkistrodon) (monovalent)	Thailand	Queen Saovabha Institute, Bangkok
Russell's viper (monovalent)	Thailand	Queen Saovabha Institute, Bangkok
Polyvalent against venoms of common cobra and Russell's viper	Thailand	Queen Saovabha Institute, Bangkok
Polyvalent against venoms of common cobra, krait, Russell's viper and saw-scale viper	India	Haffkine Institute, Bombay
Agkistrodon against venom of A. rhodostoma (monovalent)	France	Institut Pasteur, Paris
Asian cobra against venom of Naja naja (monovalent)	France	Institut Pasteur, Paris
Russell's viper	Germany	Behringwerke AG, Marburg/Lahn
Polyvalent against venoms of Agkistrodon rhodostoma, Bungarus fasciatus, and Naja naja	Indonesia	Perusahaan Negara Pasteur, Bandung
Polyvalent tiger snake neutralizes venom of tiger snake, death adder, Australian copperhead, common brown snake, red bellied black snake and the taipan	Australia	Commonwealth Serum Laboratories, Melbourne

Table 21 (Cont'd)

Antivenins	Country	Producer
Sea snake - produced with the venom of Enhydrina shistosa	Australia	Commonwealth Serum Laboratories, Melbourne
Malayan pit viper (Ancistrodon rhodostoma)	Australia	Commonwealth Serum Laboratories, Melbourne

While each of the venoms listed above may be presumed to be effective against the venom with which it was prepared, much remains to be learned about the paraspecific effectiveness of these products. Preliminary results again have shown that relationship of snake species is not always a good criterion for estimates of paraspecificity of antivenins. This was exemplified by a neutralization test conducted to determine effectiveness of various antivenins against venoms of the common cobra, Naja naja, from two localities and venom of a subspecies of the common cobra from Luzon. Results of this test are given in Table 22.

The type of information given above is absolutely essential if troops in the field are to be provided with adequate treatment in the event of snakebite. Use of an ineffective product for treatment of cobra or krait bite particularly could result in death of the patient within a few hours.

Publications:

WERLER, J. E. and KEEGAN, H. L., 1963. Venomous Snakes of the Snakes of the Pacific Area. Tenth Pacific Sci. Cong. 219-325.

WHITTEMORE, F. W., KEEGAN, H. L., FITZGERALD, C. M., BRYANT, H. A. and FLANIGAN, J. F., 1963. Studies of scorpion antivenins. 2. Venom collection and scorpion colony maintenance. Bull. Wld. Hlth Org. 28(4):505-511.

WHITTEMORE, F. W. and KEEGAN, H. L., 1963. Medically important scorpions in the Pacific Area. Tenth Pacific Sci. Cong. 107-110.

Table 22. Neutralization of Three Cobra Venoms by Five Antivenins[1]

Antivenins	Venoms[2]		
	Common Cobra *Naja naja* (from India)	Common Cobra *Naja naja* (Malayan yellow color phase)	Luzon Cobra *Naja n. philippensis* (from Luzon, P.I.)
German polyvalent cobra	X	X	X
French African cobra	X	X	X
French Asian cobra	X	X	X
Australian Malayan cobra	X	X	X
Philippine Luzon cobra	0	0	X

[1] Judged by survival at 24 hours of 14-18 g white mice given intraperitoneal injections of 5 LD_{50}'s of venom in saline, plus 0.25 ml antivenin. Venom solutions and antivenins were mixed and allowed to stand for one hour at room temperature before injecting.

[2] Symbols: X - all mice survived, 0 - no mice survived.

MEDICAL ZOOLOGY DEPARTMENT

During FY 1963, the Department of Medical Zoology had the dual mission of furnishing clinical laboratory diagnostic services for medical installations of the United States Security Forces and of conducting research on parasitic diseases of military medical importance. Routine procedures included identification of parasitic helminths, protozoa and various other zoologic specimens, bio-assays of gonadotropic hormones, the detection of parasitic contamination of vegetables and of soils in which they were grown, and the production of skin test antigens. The major research efforts were directed toward the diagnosis and control of trematode infections although studies were pursued on other aspects of parasitic diseases.

Other aspects of departmental functions are concerned with the training of laboratory officers and enlisted men assigned to various military medical units in the WESTPAC area, and also in supplying various parasitic specimens for laboratory training programs.

Occasionally this department is able to assist other military and civilian laboratories in meeting parasitologic problems by furnishing parasitic specimens, host tissue and data pertinent to parasitic infections in the WESTPAC area.

Certain phases of the investigative program are conducted in collaboration with medical personnel of the Naval Medical Research Unit No. 2 (NAMRU-2), Taiwan, the Department of Health of Yamanashi Prefecture and the Yamanashi Medical Research Institute, Japan.

Summaries of routine activities for FY 1963 are presented in Tables 1-3. Inasmuch as stool specimens ordinarily require more than one type of examination, the numbers given in the tables represent the total number of procedures rather than number of specimens.

INTERBREEDING OF THE FOUR SPECIES OF ONCOMELANIA AND THE INFECTIVITY OF THE RESULTING HYBRIDS TO THE FOUR GEOGRAPHICAL STRAINS OF SCHISTOSOMA JAPONICUM

Description:

The Annual Progress Report for FY 1962 outlines the purpose of this study, method of rearing the four species of Oncomelania snails in the laboratory and procedures for establishing oncomelanid hybrid snail colonies.

Progress:

An adequate number of laboratory-reared Oncomelania hupensis, the intermediate snail host of the Chinese strain of Schistosoma japonicum, has been produced so that the life cycle of this strain of S. japonicum can now be maintained in the laboratory. (Doctor H. F. Hsu, State University of Iowa, Iowa City, graciously supplied a few infected O. hupensis for this purpose.)

Table 1. Number and Type of Routine Procedures Performed on Specimens by Source (1 July 1962 - 30 June 1963)

Work performed	Source				Total
	Army	Air Force	Navy	Other	
Specimens received and shipped	13,146	739	98	1,887	15,870
Stool examinations	12,903	-	78	4,893	17,874
Occult blood	453	-	-	-	453
Scotch tapes	316	-	-	-	316
Animal stool examinations	2,403	-	3	-	2,406
Soil examinations	3,694	70	-	-	3,764
Vegetable examinations	445	568	-	-	1,013
Frog pregnancy examinations	413	8	23	-	444
Gonadotropin assays	217	65	45	10	337
Parasitic specimens shipped	2,506	26	-	246	2,778
CF test and PPT	612	-	-	-	612
Antigen preparation	1,655	-	-	-	1,655
Lyophilized specimens	589	-	-	-	589
Total	39,352	1,476	247	7,036	48,111

Table 2. Number of Procedures Incidental to Special Projects

Procedures	Number
Field investigations (hours)	2,024
Approximate number snails collected	300,000
Snail colony care (hours)	8,760
Animals inoculated and autopsied	6,727
Skin tests for schistosomiasis, clonorchiasis and paragonimiasis	6
Molluscicide screening (chemicals tested)	105

Table 3. Number of Stool Examinations and Per Cent Infected with Helminths and Protozoa (1956 - 1963)

	1956	1957	1958	1959	1960	1961	1st half 1962	1963
Total Japanese examined[1]	2,034	3,554	4,279	1,148	1,255	2,594	1,139	2,043
Per cent infected	57.7	58.3	50.6	35.3	35.2	33.52	28.32	27.12
Per cent infected with helminths	49.5	49.5	41.7	23.3	22.6	21.1	17.75	14.78
Per cent infected with protozoa	21.2	19.5	18.4	15.2	18.5	18.62	14.27	15.95

Table 3. (Cont'd)

	1956	1957	1958	1959	1960	1961	1st half 1962	1963
Total Americans examined[2]	1,124	1,641	1,545	1,495	1,228	998	441	872
Per cent infected	17.7	19.1	20.4	21.7	23.9	20.37	15.76	18.46
Per cent infected with helminths	8.3	12.8	13.3	15.3	15.5	13.23	8.96	12.96
Per cent infected with protozoa	10.4	7.2	9.3	10.4	11.8	8.87	8.8	7.45

[1] "Japanese examined" includes local nationals employed by U. S. Forces and/or premarital examinations on Japanese women. Most of these individuals reside in the Tokyo area.

[2] "Americans examined" includes American military and civilian employees and their dependents, residing in the Tokyo area. Included in the American dependents' classification are wives who are Japanese or Korean nationals.

To date, F_1 oncomelanid hybrid snails resulting from the following matings have been obtained:

1. Reciprocal cross of susceptible and refractory strains of O. formosana.

2. Reciprocal crosses of both susceptible and refractory strains of O. formosana with O. nosophora, O. quadrasi and O. hupensis.

3. Reciprocal cross of O. nosophora with O. hupensis.

4. Reciprocal cross of O. nosophora with O. quadrasi.

Two hundred hybrids obtained from the reciprocal cross of O. nosophora with O. formosana (refractory) along with 100 O. nosophora and 100 O. formosana (refractory) as the control groups, were exposed to miracidia of the Japanese strain of S. japonicum. Fifteen weeks after exposure, all snails were crushed to determine their respective infection rates. Thirty O. nosophora were infected, but none of the O. formosana nor any of the hybrids were infected.

Summary and Conclusions:

The results obtained from the initial series of experiments involving the exposure of hybrid snails to the various strains of S. japonicum, suggests that hybridization within a snail colony may prove to be a practical means of biologically controlling schistosomiasis.

Experiments involving the exposure of other groups of hybrid snails to the various geographical strains of S. japonicum are in progress.

List of Publications:

Moose, J. W. and Williams, J. E.: Infectivity of hybrid snails obtained from the reciprocal cross of Oncomelania nosophora and O. formosana to the Japanese strain of Schistosoma japonicum. J. Parasit., 49: 284, 1963.

A STUDY ON THE GROWTH OF YOUNG ONCOMELANIA NOSOPHORA EXPOSED TO SCHISTOSOMA JAPONICUM

Description:

Pesigan et al. (1) reported that the growth of male and female Oncomelania quadrasi is retarded when infected with the Philippine strain of Schistosoma japonicum. The purpose of the following study was to determine the effect on growth of young O. nosophora exposed to the Japanese strain of S. japonicum.

Progress:

From a colony of laboratory-reared O. nosophora, maintained as previously reported by Moose et al. (2), snails 3-4 mm in length were sexed and divided into groups of 200 females and 200 males. These snails were subdivided into control and test groups of 100 specimens of the same sex. The mean lengths of the control and test groups of males and females were statistically identical in proportion, 3.504 mm, 3.502 mm, 3.404 mm and 3.422 mm. Test snails were exposed individually for 6 hours to 2 miracidia of the Japanese strain of S. japonicum, hatched from ova obtained from livers of infected albino mice. No miracidia remained after exposure, indicating a high degree of penetration.

Sixteen weeks after exposure all surviving snails were measured and growth comparisons were made between groups of each sex. Exposed snails were then crushed to determine their respective infection rates. Results were as follows:

Sixteen females and 19 males were infected. Six test females died during the course of the experiment. No deaths in the control females nor in either male group occurred. The difference in mortality between the test and control females, as well as between the test females and both groups of males, was significant ($P<0.05$).

Size distribution of snail lengths revealed a significant difference between female control and test groups ($P<0.01$), but not between male groups (Table 1).

Table 1. Length of Snails in Control and Test Groups After 16 Weeks

Sex	Group	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	7.0-	Total
Female	Control	0	0	11	14	19	32	24	100
	Test	5	15	28	14	9	18	5	94*
Male	Control	1	20	43	19	12	5	0	100
	Test	7	24	37	19	7	6	0	100

* 6 died during the course of the experiment

The relation between the length of the snails and the ratio of infection (number infected/number in group) is shown in Table 2. The regression coefficient within both groups is significant ($P<0.01$), since it indicates that the finding of progressively fewer infections in the larger snails was probably not by chance.

Table 2. Relation between the Length of the Snails and the Ratio of Infection (Number infected/Number in group)

Length (mm)	Females		Males	
	Ratio	Per cent	Ratio	Per cent
4.0 - 4.4	2/5	40.0	3/7	42.8
4.5 - 4.9	5/15	33.3	7/24	29.2
5.0 - 5.4	7/28	25.0	8/37	21.6
5.5 - 5.9	2/14	14.3	1/19	5.3
6.0 - 6.4	0/9	0.0	0/7	0.0
6.5 - 6.9	0/18	0.0	0/6	0.0
7.0 -	0/5	0.0	-	-

The mean length of the control females was 6.325 mm with a variance of 0.3928 mm. Test group females, which were exposed but not infected, had a mean length of 5.677 mm with a variance of 0.7023 mm. The variance ratio and the mean difference between the two groups are significant ($P=0.01$). There was no significant difference between the mean length of the uninfected males of the exposed group and the control males, which were 5.249 mm with a variance of 0.3995 mm, and 5.280 mm with a variance of 0.3059 mm, respectively.

An interesting observation was the suppression in the growth rate of female snails exposed to miracidia, yet they showed no evidence of being infected.

Acknowledgements:

Statistical evaluation of the data by Doctor Kosei Takahashi, Institute of Physical Therapy and Medicine, Faculty of Medicine, University of Tokyo, Japan, is gratefully acknowledged.

References:

1. Pesigan, T. P.; Farooq, M.; Hairston, N. G.; Jauregui, J. J.; Garcia, E.G.; Santos, A. T.; Santos, B. C. and Besa, A.A.: Studies on Schistosoma japonicum infection in the Philippines. 2. The molluscan host. Bull. WHO, 18:481-578, 1958.

2. Moose, J.W.: Williams, J. E. and Fleshman, P.: Rice cereal as sustenance for rearing Oncomelanid snails in the laboratory. J. Parasit., 48(1): 68, 1962.

List of Publications:

Moose, J. W.: Growth inhibition of young Oncomelania nosophora exposed to Schistosoma japonicum. J. Parasit., 49:151, 1963.

THE INFECTIVITY OF ONCOMELANIA FORMOSANA FROM THREE DIFFERENT AREAS OF TAIWAN TO THE FORMOSAN STRAIN OF SCHISTOSOMA JAPONICUM

Description:

Hsu & Hsu (1) reported that, in view of suggestions made by investigators of the Medical General Laboratory (406), a possibility of strain differences existed in the snail, Oncomelania formosana. Subsequent experiments revealed that snails originating from Mei Nung, Kaohsiung Hsien, Taiwan, were more resistant to infection with the Formosan strain of Schistosoma japonicum than oncomelanid snails originating in Pu Yon, Changhua Hsien (2). This finding is noteworthy since Hsu & Hsu and Captain Robert E. Kuntz of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan (3), report that S. japonicum infected snails have never been found in the Mei Nung area.

Kuntz (4) reported that O. formosana was recently discovered in the northeastern part of Taiwan, near the town of Ilan, in I-Lan Hsien. S. japonicum infected snails were also found in this new locality. (After this snail habitat was discovered, a generous collection of non-infected I-Lan snails were received and are now being reared in the laboratory.)

Geographically, I-Lan is located in the northeast corner of the island, and is compeletely isolated from other snail habitats on the western side by a high mountain range. Theoretically, it was believed that a different strain of S. japonicum might exist here and that this strain difference might be demonstrated by exposing the oncomelanid snails collected from this area to the schistosome strain found in the Changhua area.

Progress:

Several infectivity studies were conducted with the Formosan strain of S. japonicum in laboratory-reared O. formosana originally from Changhua Hsien, Kaohsiung Hsien and, recently, in the snails from the new area. Experimental methods and results of these studies are reported here.

The snails were reared in the laboratory as previously described by Moose et al. (5). The Formosan strain of S. japonicum was obtained from naturally infected Changhua snails and maintained in the laboratory employing albino mice and laboratory-reared Changhua O. formosana.

This study consisted of a series of three experiments, which were performed at different times. In each experiment, the snails were exposed overnight to varying numbers of miracidia hatched from ova obtained from livers of infected albino mice. All snails were 5 months old at the time of exposure. In the first experiment 160 snails, each from Changhua and Kaohsiung, were exposed in groups of 10 to 100 miracidia. In the second experiment, a total of 50 snails, from these

same areas, were exposed in groups of 10 to 40 miracidia. One hundred oncomelanid snails from Changhua and I-Lan were exposed in groups of 10 to 60 miracidia in the third study. All surviving snails were crushed 12 weeks after exposure to determine their respective infection rates. Results appear in Table 1.

Table 1. Results of Miracidial Exposure of the Formosan Strain of Schistosoma japonicum to Oncomelania formosana Found in Three Different Areas of Taiwan

Origin	Experiment number	No. of snails exposed	No. of miracidia exposed to each group of 10 snails	No. of snails dead	No. of snails infected	Per cent of surviving snails infected
Changhua	1	160	100	44	42	36.2
	2	50	40	0	9	18.0
	3	100	60	13	23	26.4
Kaohsiung	1	160	100	0	3	1.8
	2	50	40	0	0	0.0
I-Lan	3	100	60	0	1	1.0

It is interesting to note the high infection rates in the Changhua O. formosana as compared to rates of infection in snails from the other two areas. Results of these experiments indicate that the snails from Kaohsiung and I-Lan are exceedingly resistant to infection with the Formosan strain of S. japonicum.

The fact that infected snails have been collected from the I-Lan area and none have been collected from the Kaohsiung area poses a new problem in understanding the host-parasite relationships which apparently exist on the island of Taiwan. Hsu & Hsu introduced evidence that the Formosan strain of S. japonicum is a zoophilic strain. Their conclusion was based on studies of the strain of S. japonicum endemic to the Changhua area in Taiwan. It has been conclusively established that the Changhua strain of S. japonicum is non-human, but the category classification of the I-Lan strain is questionable.

Summary and Conclusions:

From the data obtained in this study, the status of schistosomiasis japonica, found in Taiwan, is obscure and requires further evaluation.

References:

1. Hsu, H. F. and Hsu, S. Y. Li: Schistosoma japonicum in Formosa: A critical review. Exptl. Parasit., 12:459-465, 1962.

2. Professional Report, 1960. Medical General Laboratory (406), USAMCJ, APO 343, San Francisco, California.

3. Kuntz, R. E.: Personal communication, 1962.

4. Kuntz, R. E.: Eleventh Annual Meeting of the American Society of Tropical Medicine & Hygiene, 1962.

5. Moose, J. W.; Williams, J. E. and Fleshman, P.: Rice cereal as sustenance for rearing Oncomelanid snails in the laboratory. J. Parasit., 48(1):68, 1962.

6. Hsu, H. F. and Hsu, S. Y. Li: On the infectivity of the Formosan strain of S. japonicum in Homo sapiens. Amer. J. Trop. Med., 5:521-528, 1956.

List of Publications:

Moose, J. W. and Williams, J. E.: The susceptibility of Oncomelania formosana from three different areas of Taiwan to infection with the Formosan strain of Schistosoma japonicum. J. Parasit. In press, 1963.

THE INFECTIVITY OF WHITE ONCOMELANIA NOSOPHORA TO THE JAPANESE STRAIN OF SCHISTOSOMA JAPONICUM

Description:

Ota (1) reported the finding of 10 white Oncomelania nosophora collected (May 1957) in an endemic area of schistosomiasis japonica in Yamanashi Prefecture, Japan. A laboratory colony of these snails was started at that time.

Ota (1) expressed a desire to determine whether these white snails were susceptible to infection with the Japanese strain of Schistosoma japonicum. However, he was unable to rear a sufficient number of these snails to undertake the study. Subsequently, he gave this laboratory a few specimens from his stock primarily for exposing their progeny to miracidia of the Japanese strain of S. japonicum.

Progress:

Upon receipt of Ota's White O. nosophora (2 male and 2 females) in January 1962, they were placed in an aquaterrarium and maintained as previously described by Moose et al. (2). Subsequently, the colony increased in sufficient number to permit initiation of an infectivity study.

Fifty white and 50 normally pigmented laboratory-reared O. nosophora were exposed to miracidia of the Japanese strain of S. japonicum as previously described by Moose and Williams (3). Thirteen weeks after exposure, all the snails were crushed and examined. Seventeen white and 13 pigmented snails were infected.

Summary and Conclusions:

Results of this study indicate that in their natural habitat, the predecessors of these white laboratory-reared O. nosophora could have played a role in the transmission of schistosomiasis.

References:

1. Ota, S.: On the white variated snail of Oncomelania nosophora discovered in endemic area of Schistosomiasis japonica in Yamanashi Prefecture. Jap. J. Parasit., 8:383, 1959.

2. Moose, J. W.; Williams, J. E. and Fleshman, P.: Rice cereal as sustenance for rearing Oncomelanid snails in the laboratory. J. Parasit., 48(1): 68, 1962.

3. Moose, J. W. and Williams, J. E.: Infectivity of Hybrid snails obtained from the reciprocal cross of Oncomelania nosophora and O. formosana to the Japanese strain of Schistosoma japonicum. J. Parasit., 49: 284, 1963.

List of Publications:

Moose, J. W.; Ota, S. and Williams, J. E.: The susceptibility of white Oncomelania nosophora to infection with the Japanese strain of Schistosoma japonicum. Submitted for consideration for publication in the Japanese Journal of Medical Science and Biology, 1963.

THE COLLECTION, LABORATORY MAINTENANCE AND INFECTIVITY DETERMINATIONS PERFORMED ON A RECENTLY DESCRIBED SUB-SPECIES, ONCOMELANIA FORMOSAN SHINI

Description:

The method of rearing Oncomelania formosana shini snails in the laboratory and the purpose and technics of exposing these snails to miracidia of the Japanese strain of Schistosoma japonicum have been previously described in the Annual Progress Report for FY 62.

Progress:

Several hundred laboratory-reared O. formosana shini were exposed to the Formosan and Philippine strains of S. japonicum in addition to the Japanese strain as mentioned above. Ten weeks after exposure the snails were crushed and examined. None of the snails were infected with these three strains of the parasite.

Summary and Conclusions:

Results of this study indicate that O. formosana shini are totally refractory to infection with the Japanese, Formosan and Philippine strains of S. japonicum. This is an important finding since it was previously feared that schistosomiasis japonica could become prevalent on the island of Yonakuni.

Experiments involving exposure of these snails to the Chinese strain of S. japonicum will be undertaken.

A COMPARATIVE STUDY ON THE SUSCEPTIBILITY OF YOUNG AND OLD ONCOMELANIA NOSOPHORA TO INFECTION WITH SCHISTOSOMA JAPONICUM

Description:

Data obtained from field collections of Oncomelania nosophora in Yamanashi Prefecture, Japan, significantly showed that more young rather than old snails were infected with Schistosoma japonicum (1). From evidence presented, it appeared that infection may have been due to exposure opportunity to miracidia rather than to development of an age immunity by older snails, since young snails spend a large part of life in or closely associated with water.

Progress:

The following study was performed to determine whether age per se of O. nosophora plays an important role in the susceptibility to miracidial infection with S. japonicum.

From a colony of laboratory-reared O. nosophora maintained as previously reported by Moose et al. (2), 100 eight-month-old and 100 twenty-two-month-old snails were exposed individually to four miracidia of the Japanese strain of S. japonicum. The source of the miracidia and the snail exposure time was the same as described earlier by Moose (3). Deaths in both groups of snails occurred during the sixth week of the experiment. The surviving snails were, therefore, crushed and examined at the end of the seventh week. Results appear in Table 1.

Table 1. Results of Miracidial Exposure of Schistosoma japonicum to Young and Old Oncomelania nosophora

Age (months)	No. of snails exposed	No. of snails dead	No. of snails infected	Per cent of surviving snails infected
8	100	5	40	42.1
22	100	8	39	42.3

Conclusion:

Data presented here show that no significant differences exist in the susceptibility to infection with the Japanese strain of S. japonicum between young and old O. nosophora. This finding supports the observations reported in the 1950 Professional Report (1). It was probably the opportunity to exposure, rather than difference in susceptibility, that caused more young snails to be infected in their natural habitat.

References:

1. Professional Report, 1950. Medical General Laboratory (406), USAMCJ, APO 343, San Francisco, California.

2. Moose, J. W.: Williams, J. E. and Fleshman, P.: Rice cereal as sustenance for rearing Oncomelanid snails in the laboratory. J. Parasit., 48(1): 68, 1962.

3. Moose, J. W.: Growth inhibition of young Oncomelania nosophora exposed to Schistosoma japonicum. J. Parasit., 49(1): 151-152, 1963.

List of Publications:

Moose, J. W.: A comparative study on the susceptibility of young and old Oncomelania nosophora to infection with Schistosoma japonicum. J. Parasit., In press, 1963.

MOLLUSCICIDE STUDIES - LABORATORY SCREENING TESTS

A total of 6 organic chemicals were received for molluscicide screening tests against the snail host, O. nosophora.

Recommended methods by the Expert Committee on Molluscicides of the World Health Organization, 15 November 1960, were followed in laboratory screening tests. A complete description of these methods may be found on pages 89-90 in the Medical General Laboratory (406) Annual Professional Report for 1960.

Results of these tests appear in Table 1. Three of the 6 chemicals screened were found to possess molluscicidal activities equivalent to sodium pentachlorophenate at concentrations of 1 ppm, or greater, in the immersion test. Sodium pentachlorophenate was used as a standard of comparison. This would indicate that the chemicals could serve as efficient molluscicides if applied under flooding conditions.

None of the chemicals tested were as effective as sodium pentachlorophenate in the plate test. Therefore, it can be concluded that none of these were efficient molluscicides when applied by the spray technique and that they were not active contact poisons.

FIELD PLOT DILUTION TESTS

No chemicals were submitted for this test during the past year.

THERAPEUTIC EVALUATIONS OF A SELECTED DRUG FOR THE TREATMENT OF SCHISTOSOMIASIS JAPONICA

Evaluation of new drugs for their efficacy against the disease, schistosomiasis, has for the most part been conducted on animals infected with Schistosoma mansoni because of the comparative ease with which this species of schistosome can be maintained in the laboratory. The ability to produce and maintain a large number of snails infected with S. japonicum in the laboratory, has made it possible to perform similar therapeutic studies.

Parke, Davis & Company submitted a drug, designated CI-403A, for the purpose of conducting therapeutic tests in laboratory animals infected with S. japonicum. Tests conducted on animals infected with S. mansoni revealed that CI-403A is effective against this species and that it does possess additional features which make it a useful therapeutic agent. This drug can be administered orally.

In rural or primitive areas where qualified medical personnel are unavailable to inoculate patients with prescribed drugs against Asian schistosome, oral administration is a most advantageous method of treatment. The administration of "heavy metal" drugs currently available present a decided problem by producing side effects in patients. However, recent animal experiments reveal that the drug, CI-403A, produces no undesirable side effects. For these reasons a series of animal experiments were continued to determine the efficacy of this drug against the disease, Schistosomiasis japonica.

Since previous experiments were performed in white mice and Rhesus monkeys, it was decided to use white mice of the SM strain and Macaca cyclopis monkeys for comparative purposes. The latter were readily available to this laboratory through the very generous cooperation of the Navy Medical Research Unit #2 located in Taipei.

Mouse Experiments. A total of 360 mice were divided into six major groups as follows:

Group I - Treatment was initiated 10 days prior to exposure to the cercariae.

Group II - Treatment was initiated on the day of exposure.

Group III - Treatment was initiated 10 days after exposure.

Group IV - Treatment was initiated 28 days after exposure.

Group V - Treatment was initiated 42 days after exposure.

Group VI - The drug control group was used to determine the effects of the drug on normal non-infected animals.

Each group, I thru V, were divided into 3 subgroups:

A_1 - Infection control group

A_2 - Total dosage 400 mg/kg/day

A_3 - Total dosage 300/kg/day

The chemical company submitted this drug to the laboratory in capsules. Each capsule contained 125 mgs of the active ingredient in the form of a thick oil suspension. In order to administer the drug by stomach intubation, the contents of the capsules were emptied and further diluted with corn oil, so that the final concentration could be sufficiently administered in quanities of 0.01 ml aliquot per gram of body weight daily. Since all mice were approximately the same age and had an average weight of 25 grams, an equal dosage of 0.25 ml per mouse was given twice a day. The concentration of the drug was adjusted so that the total daily dosage would average 300-400 mg per kg per day as indicated in Table 1.

Therapeutic testing was performed by filling a 0.25 ml T.B. syringe with the drug suspension and forced into the stomach of the animal. A $\frac{1}{2}$ inch 18 gauge needle was extended by placing a 2 inch piece of polyethylene intravenous tubing, with an outside diameter of 0.067 inches and inside diameter of 0.047 inches, over the end of the needle to facilitate intubation.

Each mouse from groups, I thru V, was exposed to 13 male and 13 female cercariae. Backs of the mice were shaved and the cercariae were applied to this area. A glass slide containing 26 counted cercariae was inverted and applied to the skin of the mouse. All slides were microscopically checked to ensure the presence of both male and female cercariae.

The therapeutic effects of CI-403-A in the various groups of experimental mice appear in Table. 1. A statistical analysis of these data appear in Table 2.

The results of the experiments indicate that this drug does possess a high degree of efficacy against Schistosoma japonicum in mice. Only Group IV produced questionable results. Statistical analysis confirms these observations. No animal in the drug control group died and all animals showed signs of considerable weight gains during the period of therapy. The highest rate of worm burden reduction was observed in groups I and II, which were exposed either before treatment began or on the day treatment began, indicating that this drug possesses effective prophylactic qualities. This is an important finding and of great interest to those engaged in public health and military medical problems.

Table 1. EFFECTS OF CI 403-A RX 8422 ON EXPERIMENTAL SCHISTOSOMIASIS JAPONICA IN MICE

Group number		No. of mice used	INFECTION No. of cercariae per mouse	TREATMENT WITH CI 403-A No. days (Rx)	When commenced	Total dosage mg/mouse/day	No. of mice dead	No. of mice necropsied	RESULTS OF NECROPSY Location of worms recovered LIVER Number	LIVER Per cent	Mes/hep/port veins Number	Mes/hep/port veins per cent	TOTAL Number	TOTAL Per cent	Mean No. of worms	Per cent reduction of worms
	A_1	20	26	-	-	-	7	13	8	0.23	171	50.5	179	53.0	13.8	
G I	A_2	20	26	28	10 days prior	400 mg	0	20	10	0.5	0	0	10	1.9	0.5	96.4
	A_3	20	26	28	to exposure	300 mg	0	20	45	8.7	4	0.8	49	9.4	2.5	81.9
G II	A_1	20	26	-	-	-	3	17	6	1.4	225	50.9	231	52.2	13.6	
	A_2	20	26	28	One day of	400 mg	2	18	7	1.5	0	0	7	1.5	0.4	97.1
	A_3	20	26	28	exposure	300 mg	2	18	11	2.3	0	0	11	2.3	0.6	95.4
G III	A_1	20	26	-	-	-	3	17	26*	5.9	223	50.5	251	56.8	14.8	
	A_2	20	26	28	10 days after	400 mg	1	19	33	6.7	8	1.6	41	8.7	2.1	85.7
	A_3	20	26	28	exposure	300 mg	2	18	29	6.2	4	0.9	33	7.1	1.8	87.8
G IV	A_1	30	26	-	-	-	9	21	7	1.3	166	30.4	173	31.7	8.2	
	A_2	20	26	28	4 weeks after	400 mg	5	15	44	11.3	10	2.6	54	13.8	3.6	56.1
	A_3	20	26	28	exposure	300 mg	7	13	28	8.3	9	2.7	37	10.9	2.8	65.9
G V	A_1	30	26	-	-	-	7	23	45	7.5	188	31.4	233	39.0	10.1	
	A_2	20	26	28	6 weeks after	400 mg	4	16	18	6.7	4	9.7	22	5.3	1.4	86.1
	A_3	20	26	28	exposure	300 mg	3	17	20	4.5	5	1.1	25	5.7	1.5	85.1
Drug Control		20	-	28	-	400 mg	0	20	-	-	-	-	-	-		
Drug Control		20	-	28	-	300 mg	0	20	-	-	-	-	-	-		

* 2 worms found in lungs

Table 2. Statistical Analysis of the Results Recorded in Table 1.

	Comparison of degree of freedom					
		Mean Diff.	Mean Var.	Fa	DF	Significance
	$A_1 - A_2$	3.25	0.4579	181.74	31	P<0.001
G I	$A_1 - A_3$	2.30	0.6676	61.72	31	P<0.001
	$A_2 - A_3$	0.95	0.5473	16.49	38	P<0.001
	$A_1 - A_2$	3.35	0.3702	260.04	33	P<0.001
G II	$A_1 - A_3$	3.13	0.3649	234.72	33	P<0.001
	$A_2 - A_3$	0.22	0.3497	12.68	34	P<0.001
	$A_1 - A_2$	2.61	1.0559	487.45	34	P<0.001
G III	$A_1 - A_3$	2.67	0.5724	214.59	33	P<0.001
	$A_2 - A_3$	0.06	0.7395	0.45	35	None
	$A_1 - A_2$	1.07	0.6623	15.13	34	P<0.001
G IV	$A_1 - A_3$	1.24	0.5790	21.56	32	P<0.001
	$A_2 - A_3$	0.17	0.6658	2.64	26	None
	$A_1 - A_2$	2.26	0.5047	95.49	37	P<0.001
G V	$A_1 - A_3$	2.06	0.3983	104.15	38	P<0.001
	$A_2 - A_3$	0.19	0.6374	4.03	31	None

MOLLUSCICIDE STUDIES - LABORATORY SCREENING TESTS

A total of 6 organic chemicals were received for molluscicide screening tests against the snail host, O. nosophora.

Recommended methods by the Expert Committee on Molluscicides of the World Health Organization, 15 November 1960, were followed in laboratory screening tests. A complete description of these methods may be found on pages 89-90 in the Medical General Laboratory (406) Annual Professional Report for 1960.

Results of these tests appear in Table 1. Three of the 6 chemicals screened were found to possess molluscicidal activities equivalent to sodium pentachlorophenate at concentrations of 1 ppm, or greater, in the immersion test. Sodium pentachlorophenate was used as a standard of comparison. This would indicate that the chemicals could serve as efficient molluscicides if applied under flooding conditions.

None of the chemicals tested were as effective as sodium pentachlorophenate in the plate test. Therefore, it can be concluded that none of these were efficient molluscicides when applied by the spray technique and that they were not active contact poisons.

FIELD PLOT DILUTION TESTS

No chemicals were submitted for this test during the past year.

Table 1. Results of Molluscicide Screen Tests Using Various Compounds

Chemical	Immersion Test							Plate Test			
	10.0 ppm	5.0 ppm	1.0 ppm	0.5 ppm	0.25 ppm	0.125 ppm	0.0625 ppm	1,000 ppm	100 ppm	10 ppm	1.0 ppm
	Average per cent mortailty										
Permatox 10-SA-1	100 (100)*	100 (100)	93 (80-100)	50 (0-100)	50 (0-10)	55 (10-100)	0	100 (100)	36 (0-90)	7 (0-20)	0
Permatox 10-SA-2	100 (100)	100 (100)	93 (90-100)	50 (0-100)	30 (0-60)	5 (0-10)	0	93 (80-100)	57 (0-100)	13 (0-40)	0
Permatox 10-SA-3	100 (100)	100 (100)	93 (90-100)	90 (0-90)	0	0	0	100 (100)	30 (0-60)	10 (0-20)	0
Permatox 10-SA-4	100 (100)	93 (80-100)	3 (0-10)	0	0	0	0	100 (100)	20 (0-50)	20 (0-50)	0
Permatox 10-SA-5	100 (100)	96 (90-100)	40 (30-60)	0	0	0	0	100 (100)	50 (10-80)	0	0
Zylobrite	96 (90-100)	96 (90-100)	70 (20-100)	0	0	0	0	100 (100)	70 (50-100)	13 (0-20)	0
DDP	96 (90-100)	100 (100	100 (100)	100 (100)	35 (0-70)	0	0	100 (100)	93 (80-100)	60 (0-100)	0
Control (H_2O)	40 (0-100)	3 (0-10)	3 (0-10)	0	0	0	0	13 (0-20)	3 (0-10)	0	0

* Range of per cent is shown in parenthesis.

Four tests were performed on each dilution

MONKEY EXPERIMENTS

A total of 14 monkeys were divided into 8 groups as follows:

Group	Monkey Number	
Infected Controls	20, 21	
I	3, 4	Exposure and treatment began on the same day in order to study the prophylactic effects of the drug.
II	7, 23	Treatment began 42 days after exposure and necropsied 14 days after treatment ended.
III	12, 22	Treatment began 42 days after exposure and necropsied 28 days after treatment ended.
IV	13	Treatment began 42 days after exposure and necropsied 42 days after treatment ended.
V	15	Treatment began 42 days after exposure and necropsied 56 days after treatment ended.
VI	24, 25	Chronic infections - treatment began 70 days after exposure and were necropsied 14 days after treatment ended.
VII (Drug control monkeys)	26, 27	

With the exception of monkeys in Group VII, all animals were exposed to 200 male and 200 female cercariae on the same day. The cercariae had been previously sexed by passage through mice. The cercariae were counted out on a clean glass slide which was then inverted and placed in contact with the shaved skin of the monkey's abdomen. Following exposure, each slide was checked to ensure that all cercariae had remained on the skin. Prior to exposure, each monkey was sedated with an injection of 0.1 cc per kilo of a 1 per cent solution of Sernyl.

Series of tests were designed to study the therapeutic effects of the drug at different stages of infection and an attempt was made to give all treated monkeys an equal dosage of 150 mg per kilogram per day, with the exception of monkey #4. This proved to be far more difficult than anticipated since the monkeys had to be restrained and the drug, in capsule form, had to be placed in the esophagus to insure its retention. Also, difficulty was encountered in administering the required amount of the drug since the sealed capsules came in 2 sizes, 175 mg and 125 mg; combinations of these sizes did not permit exact administration. Detailed data of difficulties are reflected in Table 1. It should also be noted that drug levels ranged from 120 to 190 mg per kilo per day. Monkeys #3 and #4 received approximately 100 mg per kilo per day. Monkey #3 died 2 weeks after treatment began due to improper treatment methods and an autopsy revealed that some of the drug had been placed in the larynx finally getting into the lungs. The animal developed pneumonia and died.

Each animal was maintained in a separate cage, and 32 days following exposure and every other day thereafter, their stools were collected and examined for the presence of Schistosoma japonicum ova. Egg counts were made on positive stools and were recorded as numbers of eggs per 1 ml of stool.

The results of the experiment appear in Table 1.

DISCUSSION:

Monkey #21 of the infected control group began to shed eggs 39 days after exposure. Monkey #20 did not begin shedding until 67 days after exposure, which was possibly due to a light infection. Egg counts in Monkey #21 reached 1,250 per ml of stool, indicating that a large number of cercariae had developed into adults. However, 81 days after exposure, the egg count began to drop and 97 days after exposure, it reached zero. On the 109th day, a few eggs reappeared until the 126th day when they completely disappeared. Egg counts on monkey #20 never exceeded 90 per ml of stool, but there was no sharp drop in numbers as observed in monkey #21 until the 126th day. At this time the count dropped to 5 and on the 128th day reached zero and remained there until necropsy.

The results of these egg counts and necropsy findings reveal that the Macaca cyclopis species is not a very satisfactory host for the Japanese strain of schistosomiasis. Previous studies have shown that it is even more refractory to the Formosan strain. It is quite probable that this species would eliminate the infection in approximately 6 months after exposure to the cercariae. This would preclude its use in experiments which normally extend over a period of 12 weeks after exposure. However, they would still be very useful in conducting prophylactic studies.

Group I, monkey #4, did not shed eggs at any time during the experiment nor at the time of autopsy. No worms were found. Usually the developmental stage occurs in the liver. No lesions were found in the liver indicating that the developmental stage never occurred. Since monkey #3 died early in the experiment, all conclusions must be based on results obtained from one monkey. However, it appears that this drug has a decided prophylactic effect against schistosomiasis. The preliminary mouse experiments tend to substantiate this conclusion.

Group II, monkeys #7 and #23 began shedding eggs on the 39th day after exposure. Drug treatment began on the 42nd day. On the 15th day of treatment, eggs disappeared from the stools of monkey #7 and remained negative thereafter. At necropsy, no worms were found but slight liver damage was observed. On the 22nd day of treatment, eggs disappeared from the stools of monkey #23 and remained negative thereafter. Necropsy revealed no worms and very little liver damage.

Group III, monkeys #12 and #22 began shedding eggs on the 39th day after exposure. Drug therapy began on the 42nd day. Both monkeys stopped shedding eggs 18 days after treatment began. At necropsy, a few stunted worms were found in monkey #12. Microscopic examination revealed that the worms had actually degenerated and their sex could not be determined. In monkey #22, 2 worms were recovered which showed slight degeneration, but their sex could be determined. Liver damage appeared to be a little more extensive in this group than in Group II.

Table 1. Effects of CI 403-A RX 8422 on EXPERIMENTAL SCHISTOSOMIASIS JAPONICA IN MONKEYS (MACACA CYCLOPIS)

Group No.	Monkey number	MONKEY WEIGHT (kg) Initial weight	Terminal weight	Sex	INFECTION No. of cercariae/monkey	TREATMENT DATES No. days (RX)	Days post exposure	mg/kg/day	NECROPSY DATES Post treatment	Post exposure	Eggs found and hatched from feces	Location Mes. M	RESULTS OF NECROPSY Location of worms recovered Hepat. veins F	Total	Repat/port veins M	F	Total	Total number recovered	Per cent recovery
-	20 (Infected control)	3.8	4.25	M	M 200, F 200	-	-	-	-	126	Yes	3	4	7			0	7	1.75
-	21 (Infected control)	2.5	2.55	F	M 200, F 200	-	-	-	-	126	Yes	1	1	2			0	2	0.5
I	4 (Prophylactic effects)	6.5	7.25	F	M 200, F 200	28	0	80	104	132	No			0			0	0	
II	7 (2 wks post treatment)	4.25	3.8	M	M 200, F 200	28	42	160	14	84	Yes			0			0	0	
II	23 (2 wks post treatment)	3.7	3.9	F	M 200, F 200	28	42	190	14	84	Yes			0			0	0	
III	12 (4 wks post treatment)	4.75	4.75	M	M 200, F 200	28	42	150	28	98	Yes	A few stunted worms					0	A few stunted worms	
III	22 (4 wks post treatment)	4.3	3.75	F	M 200, F 200	28	42	120	28	98	Yes	1	1	2			0	2	0.5
IV	13 (6 wks post treatment)	3.2	3.45	F	M 200, F 200	28	42	160	42	112	Yes	4	4	8			0	8	2.0
V	15 (8 wks post treatment)	3.2	4.0	F	M 200, F 200	28	42	150	56	126	Yes			0	4	3	7	7	1.75
VI	24 (chronic infected monkey)	2.7	2.75	F	M 200, F 200	28	70	180	14	112	Yes	1	0	1			0	1	0.25
VI	25 (chronic infected monkey)	3.7	4.1	M	M 200, F 200	28	70	130	14	112	Yes	5	3	8			0	8	2.0
VII	26 Drug control	1.75	3.1	F		28		200											
VII	27 Drug control	1.85	3.2	M		28		200											

Group IV, monkey #13 began shedding eggs 39 days after exposure and therapy began on the 42nd day. It stopped shedding eggs 15 days after treatment began and remained negative until 109 days after exposure when eggs were again recovered from its stool. On the 112th day after exposure, it was necropsied and 8 worms were recovered.

Group V, monkey #15 began shedding eggs on the 39th day after exposure. Treatment began on the 42nd day. On the 18th day after treatment began its stool became negative and remained so until the 104th day after exposure. On the 126th day after exposure, the animal was necropsied and 7 worms were recovered.

Group VI, chronically infected monkeys #24 and #25, began shedding eggs on the 39th day of exposure. Treatment began 70 days after exposure, and on the 18th day after treatment began, their stools became negative and remained negative thereafter. They were necropsied on the 112th day after exposure and 1 worm was recovered from monkey #24 and 8 worms from monkey #25. Considerable liver damage was observed in this group.

Group VII, drug control study monkeys #26 and #27 were subjected to 28 days of therapy similar to that administered to the other groups. No side reactions were observed and both monkeys gained weight during the course of therapy.

CONCLUSIONS:

It would appear from preliminary studies that this drug is very effective as a prophylactic agent, and as a therapeutic agent in young infections of 4 weeks or less. In older infections, the treatment rate was not as spectacular indicating that either therapy should be maintained for a longer period of time or the dosage should be increased. Future tests must be done to determine which procedure can achieve a complete cure.

In general, this drug shows remarkable cure rates in infected mice and monkeys and apparently is a prophylactic agent. Since the prophylactic abilities of this drug are of great interest to public health and medical military officials, additional studies should be continued.

PATHOLOGY DEPARTMENT

The department consists of a Pathology Section and a Medical Illustration Section. The composition of the department remains the same as the previous fiscal year with a total of twelve assigned personnel.

Pathology Section

The Pathology Section serves as the Histopathology Center for the armed forces in WESTPAC. However, the medical laboratories at Tachikawa Air Force Base and the Yokosuka Naval Base continue to handle pathology within their respective services. This laboratory is therefore responsible predominatly for Army pathology in addition to that material submitted by Air Force and Navy installations located in close proximity to Army laboratories. A minor portion of the workload is attributed to pathologic examination of specimens submitted by U. S. civilian agencies, such as the Departments of Defense and State, and by Japanese civilian hospitals.

There has been no significant increase in the workload for fiscal year 1963. (Table 1). Fifty-six per cent of the specimens were received from Army installations, 7 per cent from the Air Force; 19 per cent from the Navy and Marines and 18 per cent from other sources.

Table 1. Number and Type of Cases Received and Comparison of the Workload of FY 1962 and FY 1963

Type of specimen	Number of specimens FY 62	FY 63	Percentage change FY 62 - 63
Surgical	2,230	1,856	-13
Cytology	2,294	2,968	+23
Autopsy	215	203	- 5
Frozen section	16	16	-
Miscellaneous	326	198	- 9
Total	4,953	5,241	+ 5

Surgical Pathology. The total number of surgical specimens received during the report period was 1,872, (including frozen sections). The distribution of these specimens in accordance with contributing installations is shown in Table 2.

Exfoliative Cytology. A total of 2,968 specimens were received with essentially little change in the distribution of contributing installations since the last report. The only significant change was a decrease in the number of specimens received from the 121st Evacuation Hospital from 369 to 26 specimens. (Table 3). A majority of the specimens were cervical and vaginal smears.

Table 2. Number and Per Cent of Locally processed Surgical Specimens by Contributing Installations - 1 July 1962 - 30 June 1963

Contributor	Number of specimens	Per cent
U. S. Army Hospital Zama	1,051	56.2
121st Evacuation Hospital	226	12.0
U. S. Army Hospital, Ryukyus Islands	228	12.1
Atsugi Naval Air Station	102	5.5
Chitose Dispensary	97	5.3
Taiwan Station Hospital	107	5.8
Yodogawa Christian Hospital	15	0.5
Miscellaneous	46	2.6
Total	1,872	100.0

Table 3. Number of Cytology Cases Received from Contributing Installations

Contributor	Number of cases	Per cent
U. S. Army Hospital Zama	1,825	61.0
Taiwan Station Hospital	561	19.0
American Embassy, Pakistan	85	2.9
Atsugi Naval Air Station	105	3.6
121st Evacuation Hospital	26	0.9
Chitose Dispensary	184	6.3
U. S. Army Hospital, RYIS	42	1.5
Seoul Military Hospital	85	2.9
Miscellaneous	55	1.9
Total	2,968	100.0

Autopsies

There was a marked increase in the number of autopsies processed through this department. Table 4 indicates the apparent increase in workload at the 121st Evacuation Hospital during the period covered by this report. Since few infant deaths occurred in Korea, death from unnatural causes constitutes a large percentage of the causes of death during 1963.

Research

One research project has been approved in the Department of Pathology for fiscal year 1964. The following is a brief summary of the project outlining the proposed studies:

Table 4. Number of Autopsy Cases by Source and Comparison of 1962 and 1963

Source	Number of cases 1962	1963	Percentage change
Local	14	27	48
Review			
Korea	51	126	147
Okinawa	40	50	20

The causes of death are listed in Table 5.

Table 5. Pathological Causes of Death

Causes of death	Number	Causes of death	Number
Unnatural deaths		Disease	
Vehicular accidents	23	Cardiovascular	41
Missile Wounds	36	Cerebrovascular	4
Poisonings	9	Acute obstructive emphysema	1
Drownings	4	Intraabdominal apoplexy	1
Crush Injuries	7	Glomerulonephritis	1
Falls	2	Malignant neoplasms	3
Hangings	2	Gangrenous appendix	1
Electrocutions	2	Epidemic hemorrhagic fever	2
Burns	6	Choriocarcinoma	1
Asphyxiations	11	Hypotension and anoxia	1
Blunt Injuries	1	Leukemia	1
Total unnatural deaths	103	Meningitis, acute	2
		Nutritional cirrhosis	2
Childhood (8 years and under)		Septicaemia	1
Perinatal deaths*	18	Delirium tremens	2
Congenital anomalies	1	Pulmonary emboli	2
Infections	1	Uremia	1
Convulsions	1	Guillain-Barre Syndrome	1
Pulmonary edema and congestion	1	Hemoperitoneum	2
Total childhood deaths	22	Multiple myeloma	1
		Acute bronchopenumonia	2
Undetermined	2	Pneumonia	1
		Hyperglycemia, etiology unknown	1
		Total from disease	75

Total of all causes - 202

* Perinatal deaths include immaturity, prematurity, stillbirths, erythroblastosis fetalis and hyaline membrane disease, but not congenital deformities regardless of severity.

THYROID DISEASES: A Comparative Study of Their Incidence in Japan (Tokyo) and the United States (Michigan). This project is being conducted in cooperation with various Medical Centers in the Tokyo area as well as other selected areas in Japan. A statistical study of the various thyroid disorders, as they occur in Japan, will be compared to a similar study being completed at the University of Michigan Medical Center.

Conferences and other activities. The Pathology Section continues to present monthly clinico-pathologic conferences and pathology seminars for the professional staff of the U. S. Army Hospital, Camp Zama. The Armed Forces Institute of Pathology has provided some teaching materials which have been presented during the CPCs and found to be very informative to all members in attendance.

Japanese interns from the U. S. Army Hospital, Camp Zama, receive four weeks of technical laboratory training and orientation under the direct supervision of the Chief, Department of Pathology. In addition to training in all phases of pathology, the interns are rotated through the various departments and instructed in applied laboratory methods and techniques. This program has been progressing very satisfactorily during the past year.

The Japanese-American Society of Pathologists continues to be active and this program is supported by all members of the Pathology Section. During the period covered by this report, guest speakers have addressed the group. This program has been an excellent medium for mutual exchange of information between the Japanese and American pathologists.

Medical Illustration Section

The Medical Illustration Branch, consisting of a photography and illustration activity, supports the epidemiologic, research and diagnostic services of the Medical General Laboratory (406). The Branch provides photographic support to The Surgeon General, Department of the Army, for preparation of teaching and training aids when requested. It provides clinical photographic support and a public information type of service for the local hospital and for Headquarters, United States Army Medical Command, Japan, which do not have the photographic capability of a public information office. The Branch also provides photographic support for inter-command and inter-theater projects when requested. See Table 6.

Table 6. Comparisons of Workload 1962 - 1963

Type of work	Number processed 1962	1963	Percentage change
Black and white film			
Negative processed			
35 mm	2,005	1,817	-10
4 x 5 inch	1,442	1,676	↑16
Lantern slides bound			
2 x 2 inch	287	627	↑123
3¼ x 4 inch	74	597	↑706
Prints processed			
AGO size	412	908	↑120
4 x 5 inch	881	2,333	↑165
5 x 7 inch	1,942	1,477	- 32
8 x 10 inch	7,525	5,602	- 34
11 x 14 inch	246	323	↑ 31
30 x 40 inch	2	-	-100
Direct copy	132	1,379	↑944
Motion pictures 16 mm, feet	400	-	-100
Color transparencies			
35 mm	688	1,206	↑ 75
4 x 5 inch	434	585	↑ 35
8 x 10 inch	12	-	-100
Miscellaneous			
Art illustrations	1,496	2,677	↑ 79
Print mounting	380	535	↑ 41
Total procedures	18,358	21,742	↑ 18

SEROLOGY AND BLOOD BANK DEPARTMENT

General. During the period covered by this report, July 1962 through June 1963, the Blood Bank continued to perform its mission as the major facility in WESTPAC for the procurement and storage of whole blood and blood derivatives.

This Blood Bank is responsible for routine supply of blood to all supported Army, Navy and Air Force installations in Japan and on an emergency basis to all military installations in the WESTPAC. In addition routine shipments of whole blood are made to Vietnam on a weekly basis. The number of donors drawn and units of blood used are comparable to previous reporting periods. This would indicate that there is no significant change in the number of personnel within the area served.

Collection. During the reporting period the Blood Bank obtained 3,123 acceptable units of blood from 3,274 donors. Of these, the mobile unit collected 3020 units and the fixed unit collected 103 units. Table 1 shows the number of units collected by each unit by month. Table 2 indicates the reasons for rejection of either blood donors or blood.

Table 1. Mobile and Fixed Unit Blood Collections by Month

Month	Fixed unit		Mobile unit		Total	Net
	Donors	Production	Donors	Production	donors	Production
Jul	20	20	261	246	281	266
Aug	5	5	256	251	261	256
Sep	3	3	232	221	235	224
Oct	2	2	275	266	277	268
Nov	0	0	425	408	425	408
Dec	9	9	192	181	201	190
Jan	6	6	210	200	216	206
Feb	1	1	296	285	297	286
Mar	40	40	229	215	269	255
Apr	12	12	338	315	350	327
May	0	0	203	191	203	191
Jun	5	5	254	241	259	246
Total	103	103	3,171	3,020	3,274	3,123

The bulk of the blood is drawn by mobile team operation at the outlying installations (Table 3). The United States Army Medical Command, Japan is the main source of emergency donors. Personnel donating blood are not bled on a routine basis. In this way the Blood Bank retains its capacity to cope with unforeseen emergencies without maintaining a large balance of blood on hand. This practice is in keeping with good blood banking procedures, and results in a minimal loss of blood due to outdating. Organizations are visited on a schedule commensurate with the number of personnel available as donors. Donor availability has a rough correlation with bloods used, as should be expected. See Table 4 for distribution of blood by agencies.

Table 2. Number and Type of Causes for Rejecting Blood Donors or Blood, by Month

Month	Total donors	Medical rejects[1]	Technical rejects[2]	Administrative rejects[3]	Serology rejects	Net production
Jul	281	9	3	1	2	266
Aug	261	2	3	0	0	256
Sep	235	4	4	2	1	224
Oct	277	4	4	0	1	268
Nov	425	9	6	1	1	408
Dec	201	3	8	0	0	190
Jan	216	4	6	0	0	206
Feb	297	6	4	0	1	286
Mar	269	7	6	1	0	255
Apr	350	16	6	0	1	327
May	203	6	6	0	0	191
Jun	259	10	3	0	0	246
Total	3,274	80	59	5	7	3,123

1 Medical rejects are for causes such as fever, medical history, etc.

2 Technical rejects indicate quantity not sufficient or entry failure.

3 Administrative rejects result when specific types of blood presented for donation exceed anticipated needs.

Table 3. Organizations Visited and Number of Donors Processed by the Mobile Team

Organizations visited	Number of trips	Total number of donors
Camp Zama	5	425
Fuchu	6	312
U.S. Army Depot, Japan	5	168
Johnson Air Station	3	164
Marine Air Group #11	3	285
Naval Air Station, Atsugi	6	282
Camp Oji	2	107
Tachikawa Air Base	9	595
Yokota Air Base	9	480
Camp Drake	3	192
Naval Station, Yokosuka	2	138
Kamiseya Naval Activity	1	23
Total	54	3,171

Table 4. Number of Pints of Blood Distributed to Utilizing Agencies by Month

Month	Agencies									Total
	Tachikawa AF Hosp	Johnson AF Hosp	Zama Army Hosp	Itazuki AF Hosp	Yokosuka Naval Hosp	8th Medical Field Hosp Vietnam	MGL (406)	Japan SDF Hosp	Misc Hosp	
Jul	88	43	40	31	0	20	4	0	2	228
Aug	88	24	40	26	0	25	1	0	0	204
Sep	96	44	68	15	0	25	1	14	1	264
Oct	125	34	57	25	0	20	1	12	0	274
Nov	84	31	41	17	3	20	6	11	0	213
Dec	89	38	50	18	2	25	3	14	0	239
Jan	68	22	48	13	0	17	0	12	0	180
Feb	65	61	39	20	0	16	0	14	0	215
Mar	66	55	78	12	11	16	26	9	0	273
Apr	64	20	38	14	25	20	7	13	0	201
May	93	5	78	12	34	16	13	3	0	254
Jun	86	0	60	15	13	20	9	0	3	206
Total	1,012	377	637	218	88	240	71	102	6	2,751

Special Activities. Special blood preparations and blood derivatives were made available to using facilities during the year. Plastic bags were used routinely. Platelet packs containing EDTA solution and blood packs, containing heparin as an anticoagulant, were kept on hand and made available whenever required. Plastic transfer pack units were used to divide standard size blood units into pediatric size units. Continued use and replacement of fresh frozen plasma for hemophiliacs occurred throughout the year. Concentrated red cells were furnished to using agencies upon request.

Type specific (non-pooled) single units of plasma prepared from outdated whole blood are stored at both frozen and room temperatures. During the past year 34 units of plasma were issued to hospitals supported by this Blood Bank.

Stockpiling of AB plasma from all group AB units outdated in this Blood Bank was initiated in early 1963. It is estimated that 100 units of AB plasma can be routinely accumulated per year. In case of a disaster, which might result in casualties requiring plasma as a supplement to the colloid portion of the shock unit, the ready availability of plasma would be most beneficial.

The method presently employed in the preparation of plasma from outdated blood involves the use of a sterile plastic transfer pack. The plasma is transferred from the blood pack to a 300 ml transfer pack by gravity. An aliquot is removed for sterility studies and the transfer pack is sealed.

Further studies are being conducted by this department on the effects of ultrasonic sterilization of human plasma. The purpose of these studies is to determine the efficacy of ultrasonic sterilization of plasma without the denaturation of

proteins. Ultrasonics have been used for some time for the dissolution of bacterial constituents without changes in the protein constituents of the bacteria. Malkina (1963) devised a method for ultrasonic sterilization which produced little or no change in plasma components. Studies will include electrophoresis of protein components, quantitative protein determinations and immuno-electrophoresis. It is planned to test certain plasma units with a variety of bacteria, and cultural methods to determine the efficacy of ultrasonic sterilization.

Serology

The Serology Branch continued its operation during the year as a diagnostic and reference section in which both serology and immunohematology tests were performed. Teaching activities were continued. The diagnostic capabilities include: The cardiolipin complement fixation test, the Treponema pallidum immobilization test, immunohematologic testing, and other miscellaneous tests. Table 5 reflects the number of each type of examination performed.

Table 5. Number and Type of Diagnostic and Blood Typing Examinations Performed

Type of examination	Number performed
Tests for syphilis	
Serum	
Qualitative cardiolipin microflocculation	13,107
Quantitative cardiolipin microflocculation	868
Cardiolipin complement fixation	1,312
Quantitative cardiolipin complement fixation	764
Treponema pallidum immobilization	764*
Cerebrospinal fluid	
Cardiolipin complement fixation	352
Quantitative cardiolipin complement fixation	8
Colloidal gold curve	340
Other procedures	340
Total protein	5
Immunohematologic tests	
Diagnostic procedures	1,393
Blood Bank units processed	3,139
Cold hemagglutinations	58
Heterophile antibody	518
Heterophile antibody absorptions	95

Table 5 (Cond't)

Miscellaneous procedures

C-reactive protein	67
LE preparations	56
Units of plasma prepared	74
Rose-Heller test	654
Latex fixation test for rheumatoid arthritis	654
Total	24,568

* There is no particular correlation between the number of quantitative cardiolipin complement fixation and Treponema pallidum immobilization tests performed. The totals are the same only by chance.

Syphilis Serology

Standard Tests. The cardiolipin microflocculation and complement fixation tests are used by this department for the sero-diagnosis of syphilis. The number of serologic tests performed remained constant as compared with figures of 1962. The Department, serving as a control laboratory for the entire WESTPAC, received specimens for confirmation of diagnosis from Army, Navy and Air Force installations in Korea, Okinawa and the Philippine Islands, as well as from all parts of Japan. The Department evaluated the work of local laboratories throughout the area to ensure a uniformly high level of performance. It also participated in the technical proficiency surveys conducted by the Walter Reed Army Institute of Research.

Treponema pallidum Immobilization Test for Syphilis. The Treponema pallidum Immobilization (TPI) test has been in use in the Department for seven years and a series of over 6,000 specimens has been examined by both the TPI and standard tests during that time. The TPI test is based upon the detection of antibodies in the sera of syphilitic individuals which, in conjunction with complement, immobilize and possibly kill virulent treponemes in vitro. Since living virulent treponemes are used as the antigen in this procedure, results are more specific than those obtained in the various standard tests employing antigens prepared from heterogeneous lipids. While the high cost and technical complexity of the TPI test render it unsuitable as a routine procedure, it is a most useful accessory test. It is especially valuable in the following circumstances: (1) to distinguish between biological false positive reactions and true syphilitic reactions in cases where symptoms and history do not indicate the existence of the disease, and (2) to aid in the diagnosis of cases where there is clinical evidence of syphilis which cannot be confirmed by the standard test (false negatives).

The major disadvantages are that (1) the immobilizing antibodies appear later in the course of the disease than do the standard test antibodies (reagin) so that a negative reaction may be misleading in the early stages of syphilis and (2) the immobilizing antibodies persist for a much longer period of time and do not disappear following adequate treatment of the disease as is usually the case with reagin. Consequently, the TPI test cannot be used as a criterion of cure or in diagnosing infections after the first one.

There were 469 specimens received in the Department for confirmation of diagnosis of syphilis. These specimens were subjected to the TPI, CMF and CCF tests. In over 38 per cent of the cases all three tests resulted in a reactive report. In 22 per cent of the cases, negative results were obtained in all three tests. In 1 per cent of the cases the TPI was reactive while the CFF test or both tests other than the TPI were non reactive. In 34 per cent of the cases the TPI was non reactive while the CMF or both tests other than the TPI were reactive. Twenty-two specimens (5.0 per cent) were reported as invalid. In the majority of cases unsatisfactory tests resulted from the presence of toxic substances in the serum. See Table 6.

Table 6. Percentage of Reactive and Non Reactive Results Obtained When Subjecting Blood Specimens to the Treponema pallidum Immobilization (TPI), Cardiolipin Microflocculation (CMF), and the Cardiolipin Complement Fixation (CCF) Tests

TPI	CMF	CCF	Number of specimens	Per cent	Total per cent
Reactive	Reactive	Reactive	180	38.4	
	Reactive	Non Reactive	5	1.0	
	Non Reactive	Non Reactive	0	0	39.4
Non Reactive	Non Reactive	Non Reactive	102	21.8	
	Reactive	Non Reactive	46	9.8	
	Reactive	Reactive	114	24.3	55.9
Invalid[1]			22	4.7	4.7
Total			469	100.0	100.0

1 A majority of invalid test results are attributed to the presence of toxic substances, such as antibiotics in the serum, bacterial contamination, hemolysis, etc.

The percentages obtained for the period July 1962 through June 1963 did not differ significantly from those reported in previous years. These percentages suggest that false negative and false positive figures will remain fairly constant within each test category. They further indicate and support the need for the TPI as the final aid in the clinical evaluation of biologic false positives. It should be reiterated here that the TPI test will show negative results if performed on blood drawn in the early stages of the disease. The data provided this laboratory frequently do not show which specimens were drawn during the early stages.

Miscellaneous Tests

Assistance was given other departments of the Laboratory in standardizing and testing antigens to be used in complement fixation tests for bacterial and parasitic disease. Complement fixation tests for Schistosomiasis were begun and are performed several times weekly in conjunction with a research project conducted by the Medical Zoology Department.

Immunohematologic Tests

The early diagnosis of blood group incompatibilities between spouses when the wife is pregnant is of considerable importance in alerting the obstetrician to take necessary measures to minimize complications. Numerous blood specimens from Rh negative women and from pregnant women with a history of having delivered jaundiced (erythroblastotic) infants, possibly due to ABO or other blood factors, are submitted to a battery of tests to detect both the complete (natural) and incomplete (immune) type antibodies. Some of the tests included are titration in albumin and in saline, indirect Coomb's test (indirect anti-globulin test) and absorption with Witebsky substance. The direct Coomb's test is performed on the red blood cells of infants suspected of being erythroblastotic. The Coomb's technique employs antiserums specific for human serum and will detect "blocking" or incomplete antibodies. Such antibodies, even in high concentrations, may attach to red cells and yet cause no agglutination in saline media. However, the addition of anti-human sera to this complex will bring about agglutination.

Blood collected weekly by the Blood Bank Branch is processed by procedures which include serologic tests for syphilis, ABO typing and complete Rh typing. Blood is not considered Rh negative unless it lacks the D, Du, C and E Rh factors and is, therefore, cde/cde. Saline titers are performed on all units of O blood, and those reacting at a dilution of 1:200 or higher are considered "type O high titer."

The immunohematologic problems presented were routine and included reports with medicolegal implications in reference to parenthood.

DEPARTMENT OF VETERINARY LABORATORY MEDICINE

The Department of Veterinary Laboratory Medicine, now in its eighth year as an entity, continued to increase its area of support to the over-all mission of the Laboratory. In general the year's workload was relatively heavy as indicated in Table 1. During the report period a total of 12 technical and administrative personnel comprised the staff of the Department.

Table 1. Number and Type of Specimens, Procedures, and Performance Factors

Type of specimen	Specimens received	Procedures	Performance factors
Dairy	2,625	8,582	483,236
Food	1,705	5,588	286,033
Soil and vegetable	5,004	10,008	10,008
Infectious disease	25,571	30,116	391,852
Animal blood	9,830	19,660	19,660
Animal supply	135,180	135,180	1,755,850
Total	179,915	209,134	2,946, 639

The mission of the department may be divided into three main areas: food evaluation, infectious disease studies, and diagnostic animal supply. To accomplish duties in these areas the department is divided into five Sections: Food Chemistry, Food Bacteriology, Infectious Disease Diagnosis, Veterinary Pathology, and Diagnostic Animal Supply.

During the fiscal year specimens were received from Japan, Okinawa, Korea, Iwo Jima, Vietnam, Guam and the Philippines. The number of specimens received and processed during this period far exceeded the number reported during previous years. Relatively few problems existed in shipment or receipt of specimens despite the size of the area served.

Food Chemistry: This section performs the most important function of the department, chemical and physical analysis of food subsistence items procured by Military units in the WESTPAC to determine compliance with contractual specifications. Food items, such as beverages and specialty items, which receive Food and Drug Administration or United States Department of Agriculture testing in the Continental United States, are tested by the Food Chemistry Section since the forementioned agencies do not function in the WESTPAC. A large number and variety of specimens were processed during the interval covered by this report. See Table 2.

Table 2. Food Chemistry Specimens

Type of specimen	Number	Type of specimen	Number
Beverages (non-alcoholic)	251	Meats (prepared)	18
Beverages (distilled liquors, spirits)	12	Meats (fresh or frozen)	228
Beverages (beer)	110	Meats (canned)	77
Cereal Foods	104	Oils, Fats, Waxes	25
Dairy Products (fluid)	1,976	Spices (condiments)	133
Dairy Products (solid)	649	Sugar	16
Eggs and Egg Products	12	Vegetables (canned)	197
Fish and Marine Products	419	Vegetables (frozen)	10
Fruit and Fruit Products	93	Total number analyzed	4,330

Food Bacteriology Section: Bacteriological analyses of food items, including water, was placed under departmental supervision during the third quarter of the fiscal year. Two technicians process bacteriological specimens.

Infectious Disease: The Wilhite-Bohls wet impression stain was used in examination of rabies suspect material. Results were reported without delay to the submitting installation. As an additional safety measure, the fluorescent antibody test was performed in all cases where human exposure had occurred. Results were only reported if they differed from the initial wet impression examination. After completion of mouse inoculation tests using the rabies suspect material, a final report was rendered thirty days after receipt of the specimen. The final report contained results of the wet impression smear, fluorescent antibody technique and mouse inoculation tests. See Table 3.

Table 3. Rabies Examinations

Origin	Number submitted	Number positive
Japan	47	0
Korea	40	5
Okinawa	27	1
Iwo Jima	1	0
Vietnam	2	0
Total	117	6 (all dogs)

Machiavello's stain and mouse inoculations were used in examination of psittacosis suspected material. See Table 4. No serum neutralization tests for rabies antibody titer were requested during the year.

Table 4. Psittacosis Examinations

Origin	Number submitted	Number positive
Japan	10	2
Okinawa	1	0
Total	11	2

Veterinary Pathology: More than fifty specimens were received during the year for histo-pathological examination. Twenty-four autopsies were performed on animals. See Table 5. Reports were rendered directly from the Department of Veterinary Laboratory Medicine. In many cases consultation or review was obtained from the Pathology Department (MGL (406)) or from the Armed Forces Institute of Pathology. The predominant finding was a high incidence of parasitism, especially Dirofilariae, and a high incidence of pneumonia. Poisoning was suspected in many animal deaths, but tests for heavy metals, insecticides, or other toxic substances were negative in most instances. Tissues, gastric, and intestinal contents were submitted to the Chemistry Department for toxicological analysis in all instances where poisoning was suspected.

Diagnostic Animal Supply: This section provided animals and animal blood by breeding and by procurement for use by this Department, as well as for use by other Departments of the Laboratory. See Table 6. Supervision of all laboratory animals was provided to ensure that all departments breeding animals adhered to sound principles of animal care and sanitation in biological testing or research. The section also provided assistance in administering test material to laboratory animals.

The facility has six rooms on the fourth floor of the laboratory for rearing and maintaining animals. Plans were initiated to augment the facility by addition of new animal rooms to include space for primates and consolidation of all breeding rooms into one area.

Animals issued during the year were valued at nearly eight thousand dollars. Laboratory-reared animals were in general good health and immediately available. Since the majority of animals were reared in the laboratory, transportation and quarantine problems normally connected with the purchase of animals outside of the laboratory were held to a minimum.

Table 5. Animal Autopsies (not rabies suspects)

Dogs	17
Cats	1
Pigeons	2
Pigs	3
Sheep	1
Total	24

Table 6. Number of Animals and Amount of Blood Issued

Animals	Number	Animals	Number
Hamster	127	Monkeys	25
Mice, with litters	1,223	Sheep	32
Mice, weanlings	13,034	Swine	20
Mice, adults	8,485	Sheep blood units	9,800
Rabbits	34	Horse blood units	30
Total			32,810

SOIL AND VEGETABLE ANALYSIS

A total of 3,906 soil specimens were analyzed for parasitic evidence of fecal contamination. Of this total, 264 or 6.76 per cent, were positive for ascarid ova. Sixteen specimens were positive for trichurid ova. See Table 7. Analysis of 1,098 vegetable specimens was performed. Parasitological examinations of thirty-eight specimens were positive for ascarid ova. Thirty-four of 782 samples tested, or 4.35 per cent showed presumptive bacterial evidence of fecal contamination. Soil and vegetable bacteriological examination reports were coordinated by the Department of Veterinary Laboratory Medicine with the parasitological analyses performed in the Medical Zoology Department.

Table 7. Analysis of Garden Soil for Fecal Contamination

Origin	Number of specimens	Positive for ascarid ova	Per cent positive	Positive for trichurid ova	Per cent positive
Japan	2,497	79	2.16	2	0.08
Korea	1,138	178	15.64	13	11.42
Okinawa	271	7	2.58	1	0.37
Total	3,906	264	6.76	16	0.41

DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES

During the period 1 July 1962 through 30 June 1963 an average of nineteen people were engaged in research and development activities and in diagnostic work in the Department. Work accomplished is summarized below.

Research and Development Activities

1. Subclinical Viral Hepatitis. Studies of serum glutamic pyruvic transaminase (SGPT) values on sera obtained from more than four thousand Korean and Chinese nationals (from Korea and Taiwan) were conducted. SGPT evaluations made on American troops stationed in these areas as well as in Japan were included in these studies. Biopsies from cases with an elevated SGPT activity revealed that there is a significant amount of clinically unrecognized liver disease in the WESTPAC area. Comparison of data obtained from all personnel studied in the same areas indicated that the disease process was different in Americans than in indigenous personnel. From the results obtained it can be tentatively concluded that the high amount of chronicity in viral hepatitis in indigenous personnel is not a virologic manifestation, but rather one of the host. Detailed investigation, including electron microscope, histochemical, and immunofluorescent studies, are now in progress on all tissue in an attempt to further elucidate this problem.

2. Serum Hepatitis. In a survey conducted in conjunction with personnel of the Japan Self Defense Force Hospital, forty-four patients undergoing thoracic surgery were studied to determine the significance of serum hepatitis in this group. Twenty-two of the forty-four patients were studied for the full five-month incubation period. Of this number, seven developed definite serum hepatitis and one cure was probable. Four of the group, not yet studied for the complete incubation period, also developed serum hepatitis. All cases developing hepatitis (except one) received blood known to have a high SGPT value, or received blood from a donor who had a pathologically established diagnosis of hepatitis. All cases were anicteric. Serum and tissue were obtained both pre and post infection. Electron microscope and immunofluorescent studies will be conducted on all biopsy tissue obtained.

3. Development of Fluorescent Antibody Test for Laboratory Diagnosis of Enterovirus. Attempts are underway to type unknown viral isolations by means of fluorescent labeled sera in combination pools. Hyperimmune rabbit and/or monkey sera will be made, and it is planned to concentrate the globulin fraction and conjugate this to fluorescein isothiocyanate and to test various pools of these sera against already titered viruses (Coxsackie A_9, B_1 through B_5, and Echo 1 through 18). If successful this should considerably reduce the time and effort required to identify enterovirus isolates.

4. Support has been given the Entomology Department in their studies on the overwintering mechanism of Japanese encephalitis virus.

Diagnosis of Virus and Rickettsial Diseases

The diagnostic tests available in the Department of Virus and Rickettsial Diseases are shown in Table 1. In general, tests are carried out only on cases from whom paired sera and history are received. It is preferable that isolation specimens also be available. The criterion employed for diagnosis was a fourfold rise or fall in antibody titer.

The methods currently employed for all viral diagnostic procedures, including detailed methodology involved in preparation of antigens and tissue cultures, and methodology of carrying out tests, are described in a separate standard operating procedure recently published. Requests for this document should be addressed to Commanding Officer, 406 Medical Laboratory, U. S. Army Medical Command, Japan, APO 343, San Francisco, California.

Respiratory Virus Infections

Comparison with a similar period in 1961-1962 shows a sharp decline in the number of cases diagnosed as viral infections of the respiratory system. Only in the adenoviruses and Q-fever were the number of cases diagnosed as positive comparable in number to those of the previous year.

Influenza especially reflected this trend. Only thirty-three cases were diagnosed in the 1962-1963 period contrasted with approximately 130 during the 1961-1962 period. All but eight of the thirty-three cases occurred in 1962. Twenty-eight of these cases were influenza A, two were influenza B, and two showed a rise in titer to both influenza A and B. Clinically, all cases on which histories were received showed symptoms of fever, chills, malaise, headache, myalgia, and cough. Only one case developed clinical symptoms of pneumonia. The symptoms as reported were less severe than those of the 1961-1962 period. The data on serological diagnosis of influenza are summarized in Table 2.

Respiratory virus infections other than influenza on which positive diagnoses were made are summarized in Table 3.

Throat washings for viral isolation were received on nine cases. Isolation was attempted by inoculation of monkey kidney and HeLa cell tissue cultures and of embryonated eggs. A cytopathogenic agent, probably viral, was isolated from material from Case M.A.S. (see Table 3) by inoculation of monkey kidney tissue culture with production of hemagglutinins and hemadsorbtion. This case showed a rise of antibody titer during serological testing that indicated infection by parainfluenza viruses. This agent remains unidentified.

Central Nervous System Viral Infections

The cases diagnosed as diseases of the central nervous system are summarized in Table 4. Virus isolated from throat washing material from Case M.C. (see Table 4) by inoculation of monkey kidney tissue cultures and suckling mice was identified as Coxsackie B_5. Reisolation from original material was positive.

Table 1. DIAGNOSIS OF VIRAL AND RICKETTSIAL DISEASES

PROCEDURES IN CURRENT USE
AT THE DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES
MEDICAL GENERAL LABORATORY (406)

DIAGNOSTIC TESTS AVAILABLE IN DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES
MEDICAL GENERAL LABORATORY (406)

	DAY OF ILLNESS TO OBTAIN SERUM		SPECIMENS FOR ISOLATION	DAY OF ILLNESS TO OBTAIN INFECTIOUS MATERIAL
	ACUTE	CONV.		
ADENOVIRUS	0-6	20-25	TW, TS	0-6
COXSACKIE A	0-6	10-20	TS, RS, F, CSF	0-14
COXSACKIE B	0-6	10-20	TS, RS, F, CSF	0-14
CYTOMEGALIC INCLUSION DISEASE	WHEN AVAILABLE		URINE	WHEN AVAILABLE
DENGUE 1 AND 2	0-6	20-25	WB	0-6
ECHO VIRUSES	0-6	10-25	TS, RS, F CSF	0-14
ENCEPHALOMYOCARDITIS	0-6	20-25	TS. RS, F CSF	0-14
EXANTHEMA SUBITUM (ROSEOLA INFANTUM)	0-6	20-25	TS TS, F RS, CSF	0-6
HEMADSORPTION GROUP	0-6	20-25	TS, TW	0-6
HEMORRHAGIC FEVER*	0-6	20-25	WB, URINE, PLASMA, AUTOPSY TISSUE	EARLIEST AVAILABLE
HERPES SIMPLES	0-6	20-25	TS, SWAB OF LESION, CSF	EARLIEST AVAILABLE
INFECTIOUS HEPATITIS*	0-6	14-21	F, AND AUTOPSY LIVER	WHEN AVAILABLE
INFLUENZA	0-6	10-20	TW, TS	0-6
JAPANESE ENCEPHALITIS	0-6	10-20	BRAIN (AUTOPSY)	EARLIEST AVAILABLE
LYMPHOCYTIC CHORIOMENINGITIS	0-6	20-25	CSF	0-6
LYMPHOGRANULOMA VENEREUM	0-6	20-25	NONE REQUESTED	
MEASLES (RUBEOLA)	0-6	20-25	TW, TS, F, RS	0-6
MUMPS	0-6	20-25	NONE REQUESTED	
POLIOMYELITIS	0-6	10-20	TS, RS, F	
PSITTACHOSIS-ORNITHOSIS	0-6	20-25	NONE REQUESTED	
PRIMARY ATYPICAL PNEUMONIA	0-6	20-25	NONE REQUESTED	
RABIES	0-6	20-25	BRAIN (AUTOPSY)	WHEN AVAILABLE
TRACHOMA	0-6	20-25	NONE REQUESTED	
VARICLA	0-6	20-25	PUSTULES, CRUSTS	0-14
VACCINIA	0-6	20-25	PUSTULES, CRUSTS	0-14
COE VIRUS	0-6	10-25	TW, TS	0-6
RESPIRATORY SINCITIAL VIRUS	0-6	10-25	TW, TS	0-6
CROUP ASSOCIATED VIRUS	0-6	10-25	TW, TS	0-6
RICKETTSIA	0-6	20-25	WB, BRAIN, SPLEEN (AUTOPSY)	0-4
ENDEMIC TYPHUS	0-6	20-25	WB, BRAIN, SPLEEN (AUTOPSY)	0-4
EPIDEMIC TYPHUS	0-6	20-25	WB, URINE	0-4
Q-FEVER	0-6	20-25	WB, URINE	0-4
SCRUP TYPHUS	0-6	20-25	WB, BRAIN, SPLEEN(AUTOPSY)	0-4

TESTS NOT AVAILABLE,
CAT SCRATCH FEVER
CHICKEN POX
COMMON COLD
HERPES ZOSTER
RUBELLA

NOTE: TW: THROAT WASHING
TS: THROAT SWAB
RS: RECTAL SWAB
F: FECES
CSF: CEREBROSPINAL FLUID
WB: WHOLE BLOOD

*DIAGNOSTIC TESTS ARE NOT AVAILABLE; HOWEVER, SPECIMENS ARE NEEDED FOR RESEARCH

TABLE I. ABBREVIATION USED:
CONV. CONVALESCENT

Miscellaneous Virus Infections

Miscellaneous viral infections diagnosed are summarized in Table 5.

Summary of Diagnoses

Virological diagnoses made during this period are summarized by month of onset of disease in Table 6.

Table 2. Influenza Diagnoses, 1962-1963

Case	Location	Date onset	Date sera	Reciprocal of antibody titer: Influenza A_2 CF[1]	Influenza A_2 HI[2]	Influenza B CF[3]	Influenza B HI Lee[4]	Influenza B HI Great Lakes[5]
Influenza A								
R.L.	Philippines	?	9-6-62	<8	20	8	<20	40
			9-21-62	32	20	8	<20	40
D.R.	Philippines	?	9-19-62	8	<10	8		
			10-10-62	64	<10	8		
G.S.	Philippines	7-6-62	Acute	<8	<10	16		
			Conv.	16	20	16		
J.S.	Okinawa	7-31-62	Acute	8	20	16		
			Conv.	32	160	16		
D.C.E.	Okinawa	4-28-62	Acute	16	<10	8		
			Conv.	32	160	8		
H.A.L.	Japan	3-7-62	Acute	16	<10	16		
			Conv.	64	20	16		
R.H.	Japan	?	2-27-62	<8		32		
			3-12-62	64		16		
H.D.	Japan	?	2-23-62	<8		8		
			3-9-62	64		16		
V.B.	Japan	?	2-19-62	8	<10	16		
			3-6-62	64	80	8		
A.B.	Japan	4-10-62	4-26-62	64	80	8		
			5-4-62	64	320	8		
R.A.	Japan	4-6-62	4-9-62		10			
			5-2-62		160			
A.J.S.	Japan	2-23-62	Acute	8		8		
			Conv.	32		8		
C.T.R.	Japan	3-10-62	Acute	8	<10	32		
			Conv.	64	320	32		
D.P.	Japan	2-25-62	3-2-62	8		16		
			3-15-62	64		16		
H.E.M.	Japan	3-3-62	3-5-62		40	<8		
			3-19-62		160	<8		
L.M.S.	Japan	?	1-31-63	1:10	8	1:10	8	
			2-20-63	1:10	32	1:10	16	

Table 2 (Cont'd)

Case	Location	Date onset	Date sera	Reciprocal of antibody titer: Influenza A2 CF[1]	Influenza A2 HI[2]	Influenza B CF[3]	Influenza B HI Lee[4]	Influenza B HI Great Lakes[5]
Influenza A								
A.S.	Japan	?	1-31-63	1:10	16	<10	8	
			2-20-63	1:40	64	1:10	16	
L.C.	Japan	?	1-31-63	1:40	16	1:20	8	
			2-20-63	1:20	64	1:20	16	
R.E.A.	Japan	?	1-31-63	1:10	16	<10	8	
			2-20-63	1:40	16	<10	8	
A.L.H.	Guam	4-18-62	Acute	8		32		
			Conv.	32		32		
B.M.	Guam	10-29-62	11-1-62	16	10	32	160	80
			11-26-62	≧128	40	64	160	80
C.V.B.	Guam	10-26-62	10-30-62	8	10	64	160	320
			11-20-62	≧128	40	64	160	320
J.E.	Korea	?	3-14-62		10	8		
			Conv.		160	16		
Z.G.S.	Korea	3-6-62	3-14-62	16	<10	8		
			4-2-62	32	640	8		
J.D.S.	Korea	2-22-62	2-26-62	<8				
			3-12-62	16				
T.S.	Korea	?	3-8-62	8	<10	<8		
			4-3-62	32	20	<8		
E.E.R.	Korea	?	3-8-62	16	<10	<8		
			3-22-62	32	20	<8		
C.R.M.	Korea	3-6-62	3-8-62	16	10	128		
			Conv.	64	40	64		
N.J.	Guam	3-19-63	3-20-63	<8	<20	1:16	1:40	1:80
			4-17-63	1:32	1:160	1:16	1:40	1:40
Influenza B								
J.H.	Guam	7-28-62	Acute	16	80	4		
			Conv.	16	160	64		
D.T.	Japan	?	1-31-63	<10	16	1:10	8	
			2-20-63	1:10	16	1:80	8	

Table 2 (Cont'd)

Case	Location	Date onset	Date sera	Reciprocal of antibody titer: Influenza A_2 CF[1]	Influenza A_2 HI[2]	Influenza B CF[3]	Influenza B HI Lee[4]	Influenza B HI Great Lakes[5]
Influenza A and B								
R.W.K.	Japan	2-18-63	Acute	<8	<10	<8	1:10	
			Conv.	16	<10	32	1:10	
W.B.	Japan	3-13-63	3-14-63	<8	320	<8	<10	<10
			3-28-63	≧128	2500	16	<10	1:10

1 Complement fixation (CF) test with Asian strain Flu A_2/Japan/775/60
2 Hemagglutination inhibition (HI) test with Asian strain Flu A_2/Japan/775/60 and Kaolin adsorbed sera
3 Complement fixation (CF) test with Lee strain and allantoic fluid
4 Hemagglutination inhibition (HI) test with Lee strain, influenza B, and Kaolin adsorbed sera
5 Hemagglutination inhibition (HI) test with Great Lakes strain, influenza B, and Kaolin adsorbed sera
6 Abbreviations used within this table: Conv. - Convalescent

Table 3. Respiratory Virus Infections (Other than Influenza) Diagnoses 1962 - 1963

Case	Location	Date onset	Date sera	Reciprocal of antibody titer: Adeno virus	Cold Agg.	HA-1	HA-2	CA	Coe	Resp sync	Q-fever	Symptoms
Adenovirus												
J.H.	Okinawa	2-19-62	2-20-62	<5	1-2	20	<20	<20	<10	<4	<5	Nausea, diarrhea, nasal congestion
			3-7-62	10	1-2	20	<20	<20	<10	<4	<5	
K.C.	Okinawa	1-22-63	Acute	1:5								Generalized maculopapular rash
			Conv.	1:20								
R.C.T.	Japan	2-8-62	2-14-62	<5		80	20	<20	<10	<4	<5	Fever, chills, cough, epistaxis
			4-18-62	1:10		<20	<20	<20	<10	<4	<5	
J.D.N.	Guam	2-9-63	2-13-63	5								Fever, headache, drowsiness
			3-6-63	≦1:40								
Q-Fever												
M.S.	Okinawa	?	2-19-62	<5		40	<20	40			<5	Malaise, rhinorrhea, cough
			3-1-62	<5		40	<20	40			10	
Parainfluenza												
M.A.S.	Korea	2-1-62	2-2-62	<5		40	<20	<20			<5	Fever, chills, cough, headache
			3-10-62	<5		80	40	80			<5	

1 Abbreviations used within this table: Cold Agg. - Cold agglutination, HA - Hemadsorption, CA - Croup associated, Resp sync - Respiratory syncitial, Conv. - Convalescent

Table 4. Central Nervous System Infections Diagnoses
1962 - 1963

Case	Location	Date onset	Date sera	Reciprocal of antibody titer JE CF	JE HI	Mumps	Coxsackie B_1	Coxsackie B_5	Symptoms
Japanese Encephalitis									
T.G.W.	Okinawa	8-1-62*	Acute	<5	<10				No clinical data
			Conv.	<5	40				
T.U.	Okinawa	10-4-62	Acute	20	40				Fever, headache, nausea, vomiting
			Conv.	80	320				
W.C.N.	Okinawa	9-15-62*	Acute		10				No clinical data
			Conv.		80				
K.K.	Okinawa	10-5-62	Acute	5	20				Fever, vomiting, headache, convulsion
			Conv.	20	160				
R.P.C.	Okinawa	9-7-62*	Acute	<5	20				No clinical data
			Conv.	5	640				
J.H.G.	Okinawa	?	7-19-62	5	40				No clinical data
			8-6-62	10	160				
T.G.	Okinawa	10-6-62	Acute	10	160				Fever, vomiting, excited
			Conv.	80	1280				
T.I.	Okinawa	10-9-62	Acute	5	160				Fever, headache, mentally disturbed
			Conv.	80	2560				
S.H.	Okinawa	10-7-62	Acute	5	20				Fever, headache, vomiting
			Conv.	160	160				
S.I.	Okinawa	10-8-62	Acute	<5	20				Fever, convulsion, mentally disturbed
			Conv.	40	80				
R.Q.**	Okinawa	?	6-16-62	16	160	<4			Fever, vomiting, lethargy, nuchal rigidity
			7-10-62	<4	10	<4			
T.R.G.	Okinawa	6-17-62*	6-21-62	<4	40				Fever, headache, lethargy
			7-19-62	16	640				
			8-17-62	32					
Coxsackie B_1									
J.W.	Philippines	?	12-7-62				1:320		Severe pain in hypochondria region and left lower lung field, malaise, cough
			12-8-62				1:10		

Table 4 (Cont'd)

Case	Location	Date onset	Date sera	Reciprocal of antibody titer: JE CF	JE HI	Mumps	Coxsackie B_1	Coxsackie B_5	Symptoms
Coxsackie B_5									
H.M.	Japan	5-5-62	Acute					< 4	Fever, cough, pleuritic pain
			6-28-62					1024	
M.C.	Japan	4-25-62	5-1-62					< 5	Hoarseness, fatigue, myalgia headache, cough, fever diaphoresis
			5-24-62					1:160	
Mumps									
K.T.	Japan	2-7-63*	Acute			< 4			No clinical data
			Conv.			1:32			
P.T.	Japan	?	2-12-63			1:4			No clinical data
			Conv.			1:32			
S.S.	Japan	2-7-63*	Acute			< 4			No clinical data
			Conv.			1:64			
M.K.	Japan	2-7-63*	Acute			< 4			Parotid gland swollen and tender
			Conv.			1:32			
F.S.	Korea	1-1-63	1-5-63			1:4			Pain in jaws, bilateral swelling, headache, cough
			1-19-63			≦1:64			
A.B.	Philippines	3-9-63	Acute		< 10	< 4			Diarrhea, vomiting, lethargy, nuchal rigidity
			Conv.		< 10	1:8			
B.W.H.	Korea	?	4-24-63			1:10			No clinical data
			5-6-63			≦1:64			

* Approximate dates.
** Results indicate that sera were reversed.

Table 5. Miscellaneous Virus Infections Diagnoses, 1962 - 1963

Case	Location	Date onset	Date sera	Reciprocal of antibody titer PLV	Mumps	Herpes	Sumptoms
Lymphogranuloma Venereum							
W.J.A.	Taiwan	?	5-1-62	<5			Swelling on groin progressing to nodes with fistulation
			5-17-62	10			
J.N.	Japan	?	9-12-62	10			No clinical data
W.B.E.	Japan	?	1-14-63	1:20			No clinical data
J.G.	Okinawa	8-17-62	8-31-62	≦40			Lymphadenopathy
G.E.A.	Okinawa	?	8-16-62	20			Inguinal adenopathy
W.A.T.	Okinawa	?	2-26-63	≦40			Inguinal adenopathy
Herpes Simplex							
J.N.	Japan	3-24-62	Acute	8	4	8	Vertigo, vomiting, mild fever
			Conv.	8	<4	32	
L.S.	Japan	?	2-12-63	<8	<8	<8	Lethargy, weakness
			3-12-63	1:8	<8	1:16	

Table 6. Summary of Virologic Diagnoses, 1962 - 1963

	Month of onset of symptoms																
	1962											1963					
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Total
Respiratory virus infections																	
Influenza A	1	7	5	3	1	2		1	2		2			4	1		29
Influenza B							1							1			2
Influenza A and B													1		1		2
Adenovirus	1	1												2			4
Q-Fever		1															1
Parainfluenza	1																1
Central nervous system																	
Japanese Encephalitis						1	1			10							12
Coxsackie B_1												1					1
Coxsackie B_5				1	1												2
Mumps												1	4		1	1	7
Miscellaneous viral infections																	
Lymphogranuloma Venereum				1			1	2				1		1			6
Herpes Simplex					1									1			2
Total Number of Diagnoses																	69

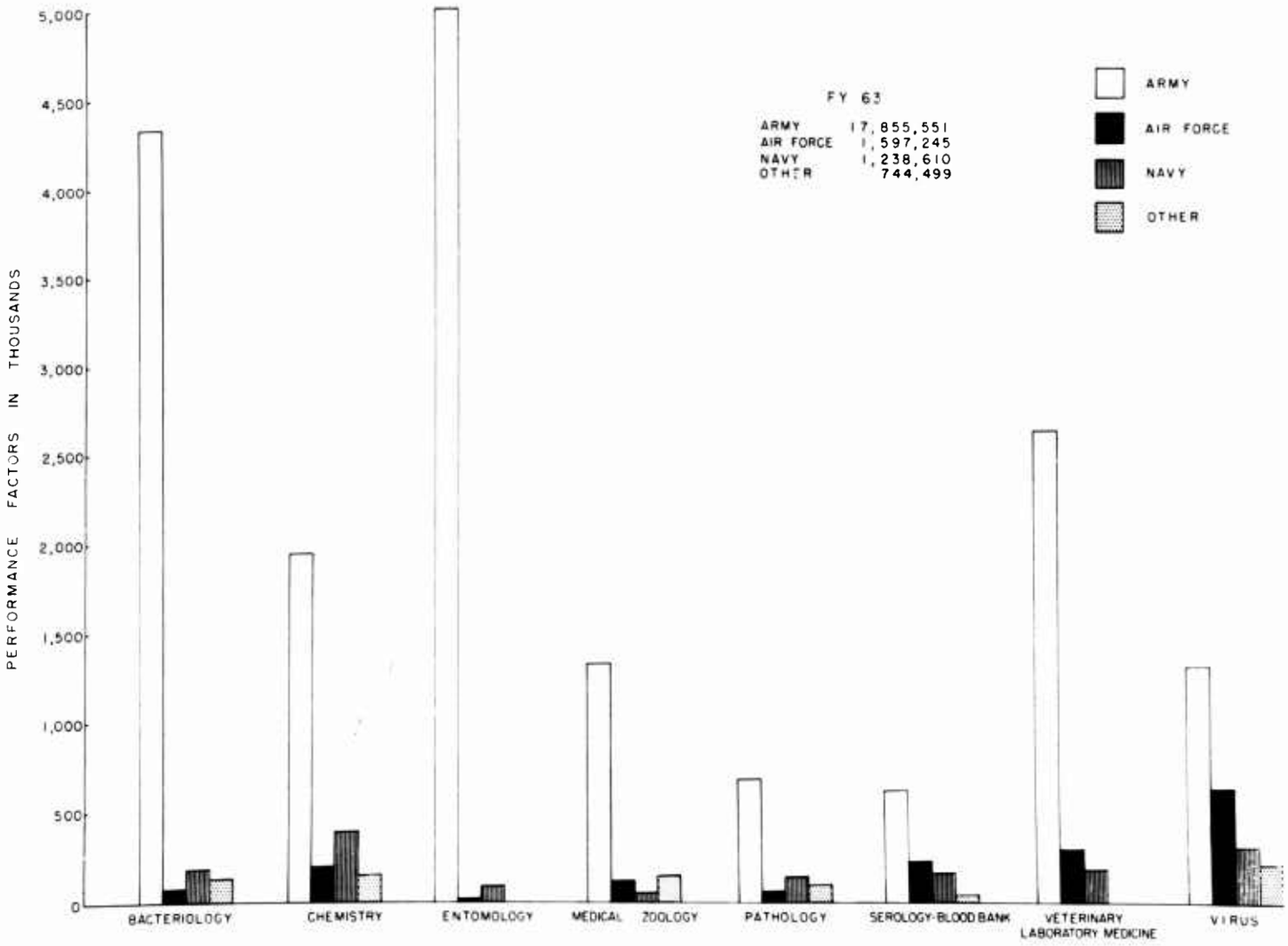
APPENDIX

PERFORMANCE FACTORS

Following are charts reflecting performance factors for each department for the year and for each department by month for the year.

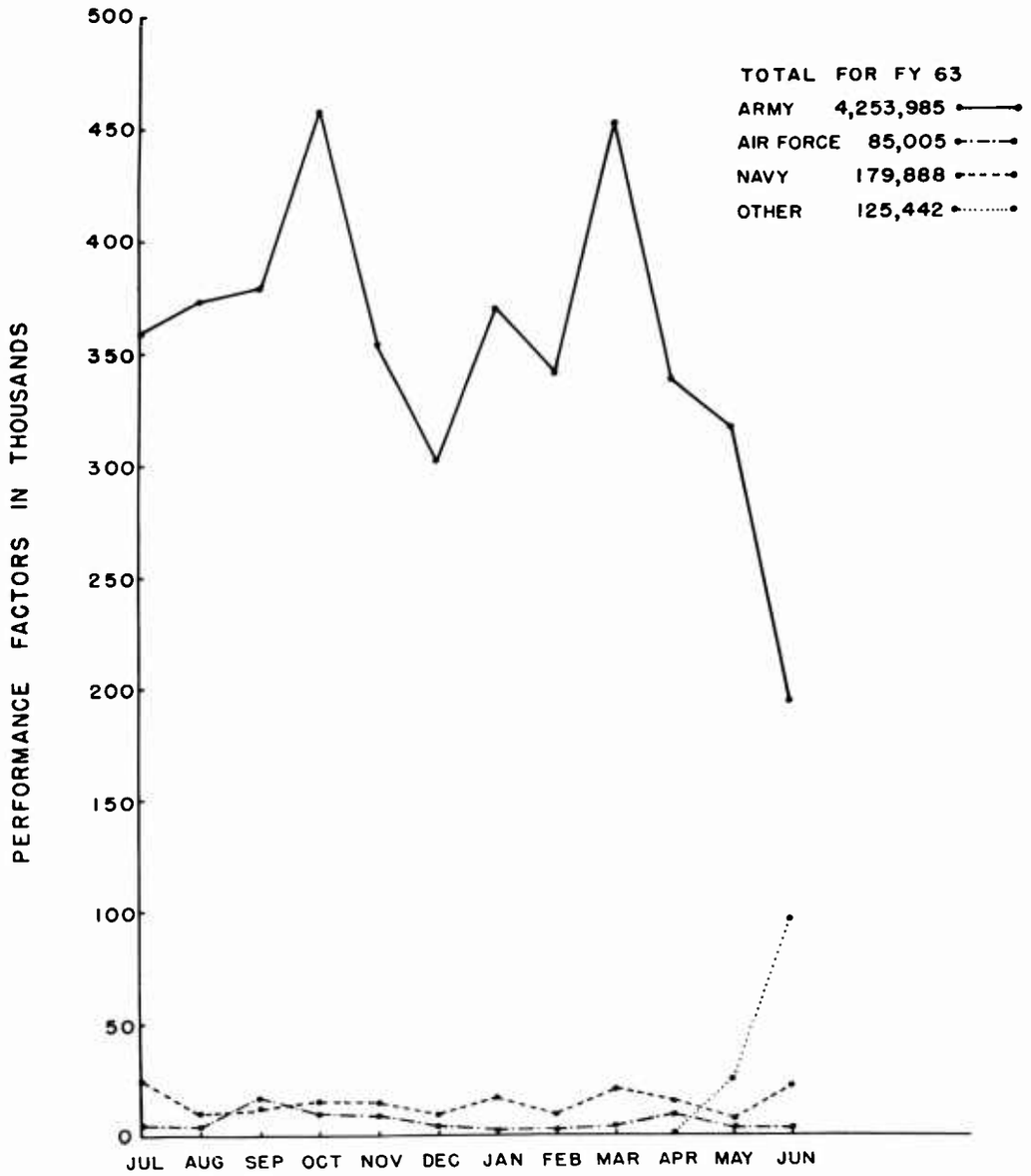
PERFORMANCE FACTORS FOR FY 63 BY DEPARTMENT AND SERVICE

MEDICAL LABORATORY (406)



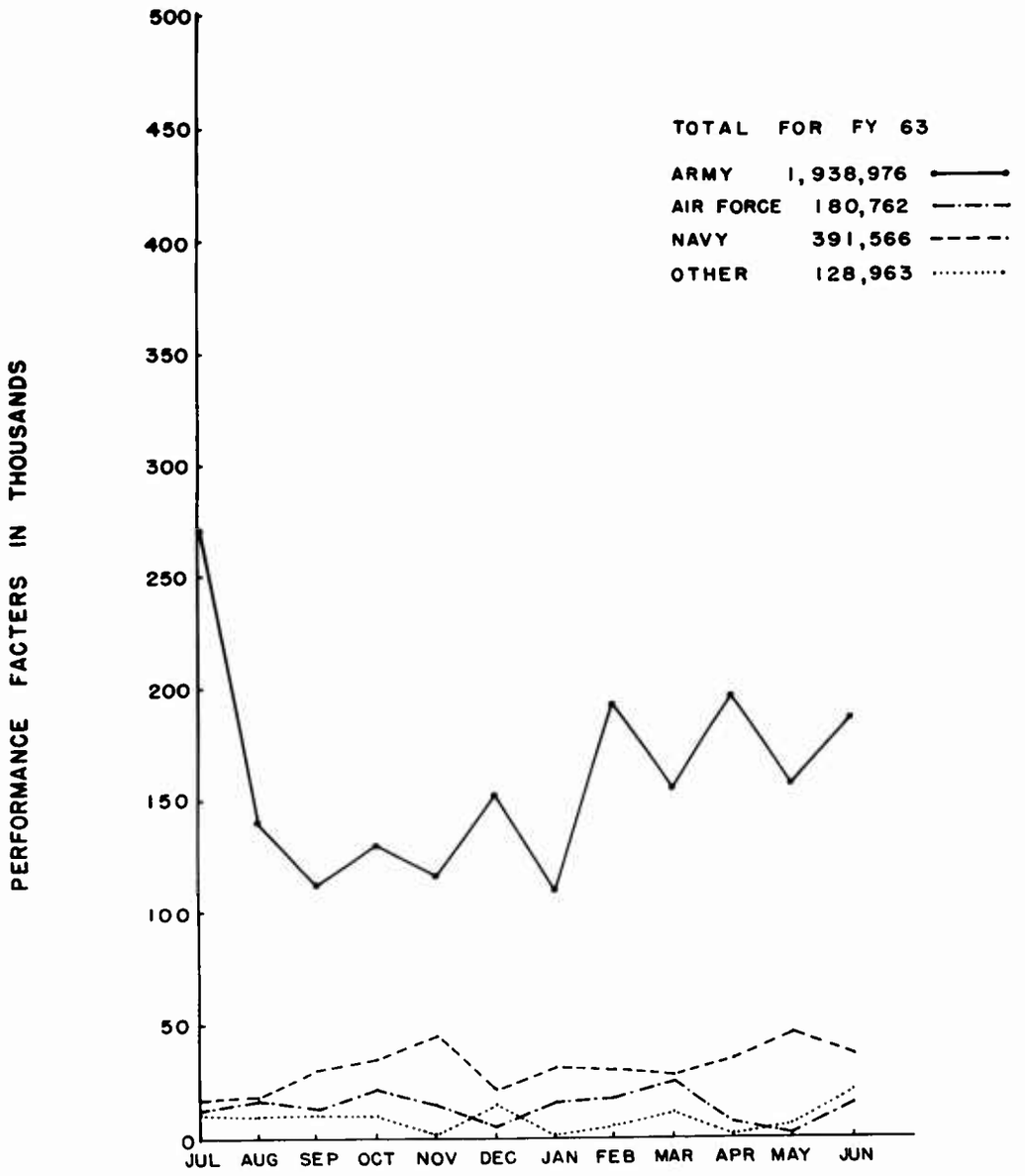
DEPARTMENT OF BACTERIOLOGY

PERFORMANCE FACTORS BY MONTH AND SERVICE



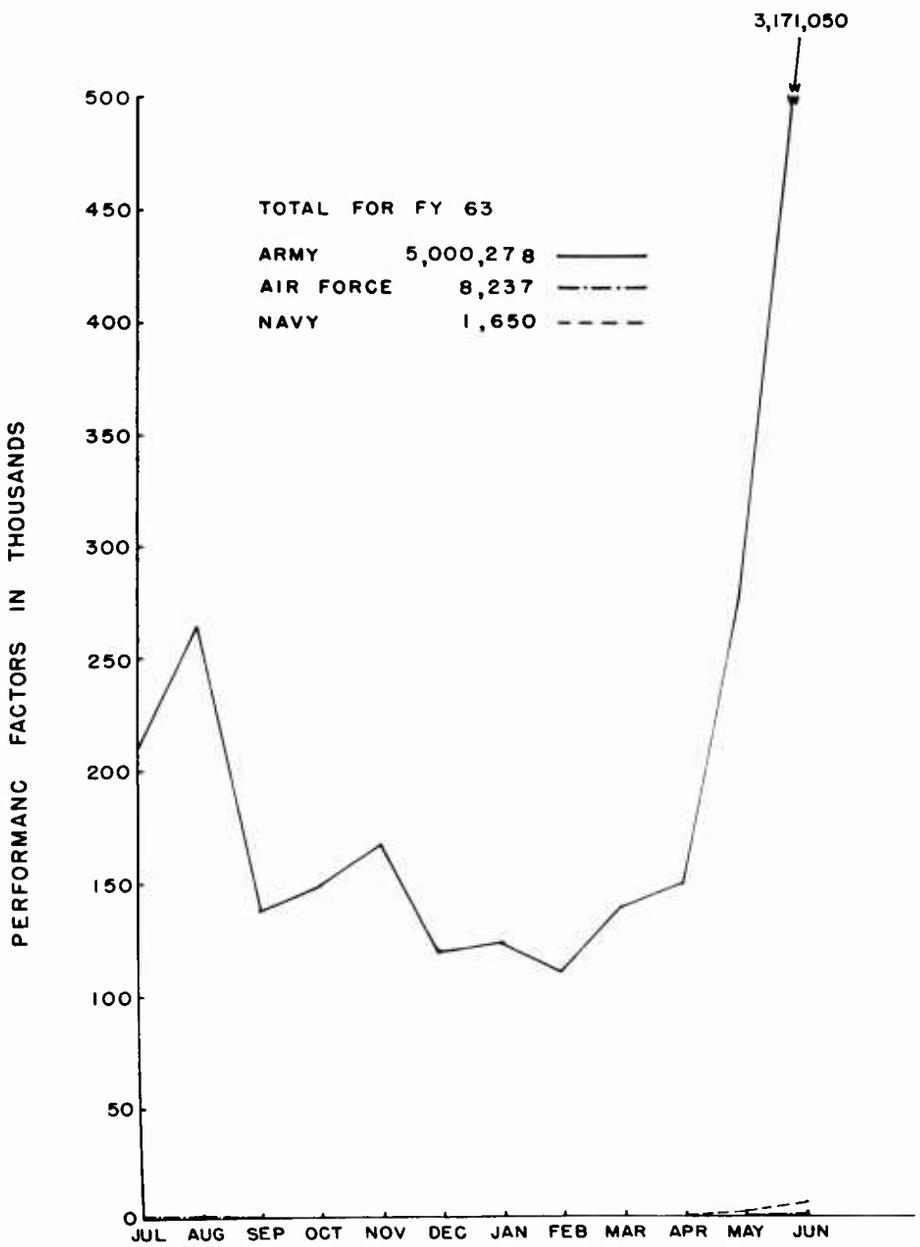
DEPARTMENT OF CHEMISTRY

PERFORMANCE FACTORS BY MONTH AND SERVICE



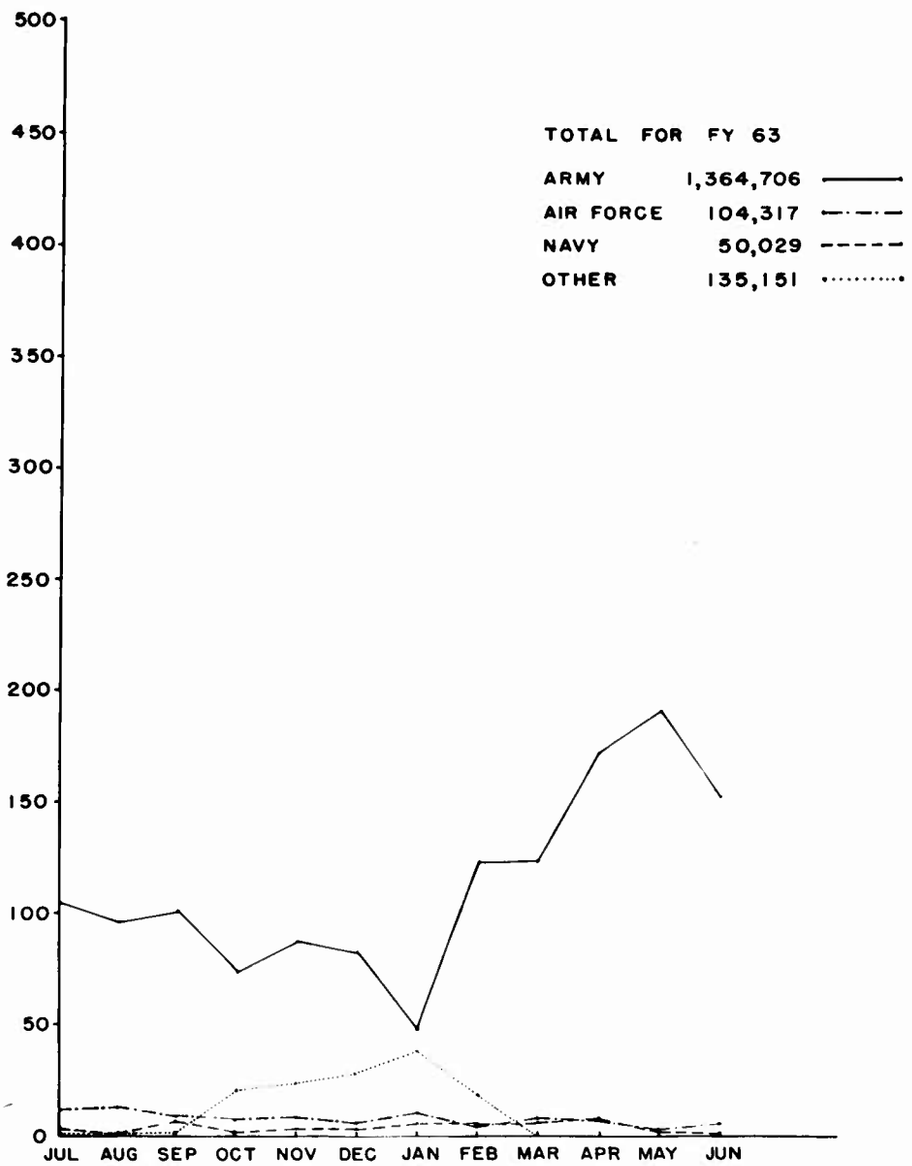
DEPARTMENT OF ENTOMOLOGY

PERFORMANCE FACTORS BY MONTH AND SERVICE



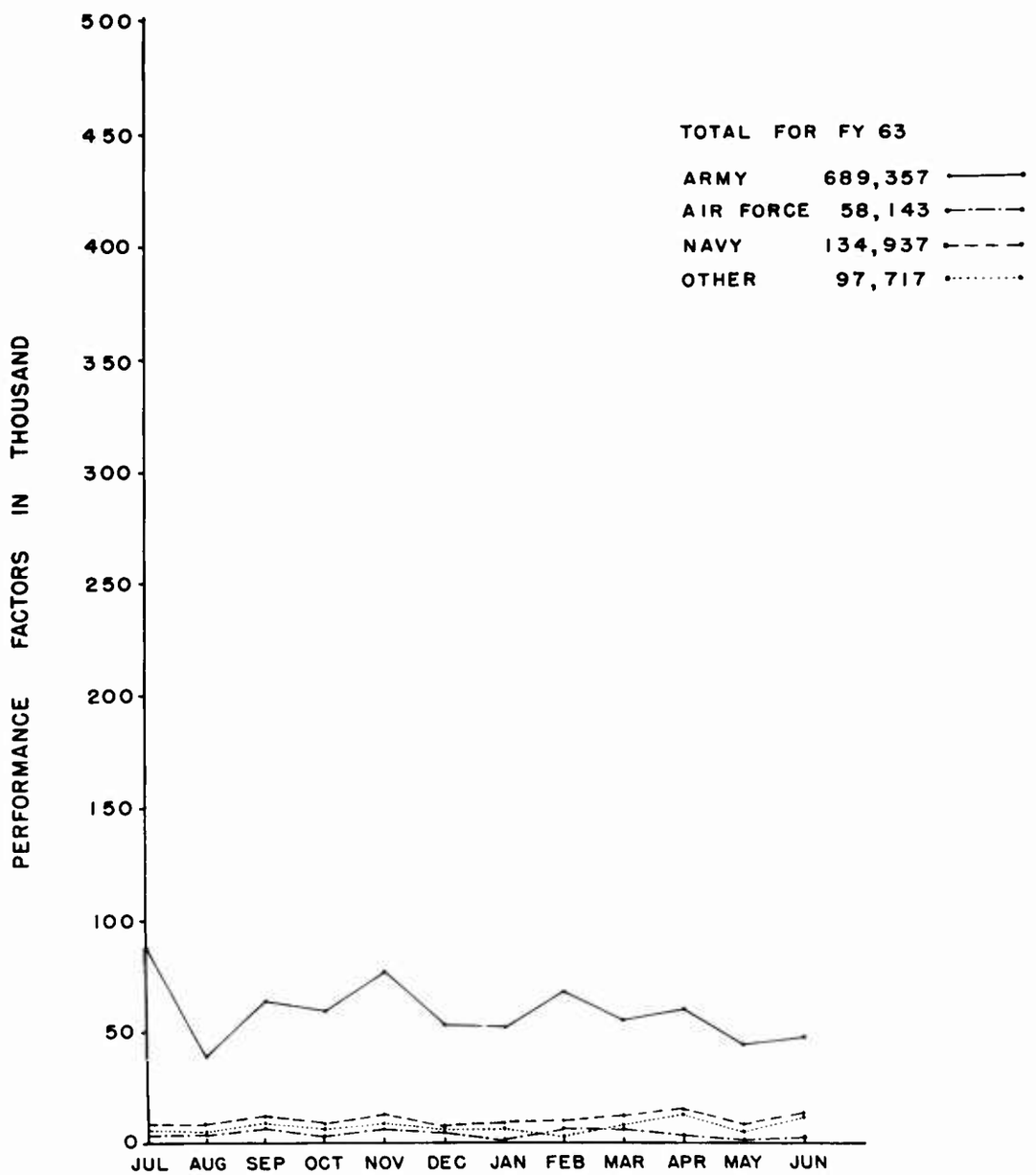
DEPARTMENT OF MEDICAL ZOOLOGY

PERFORMANCE FACTORS BY MONTH AND SERVICE



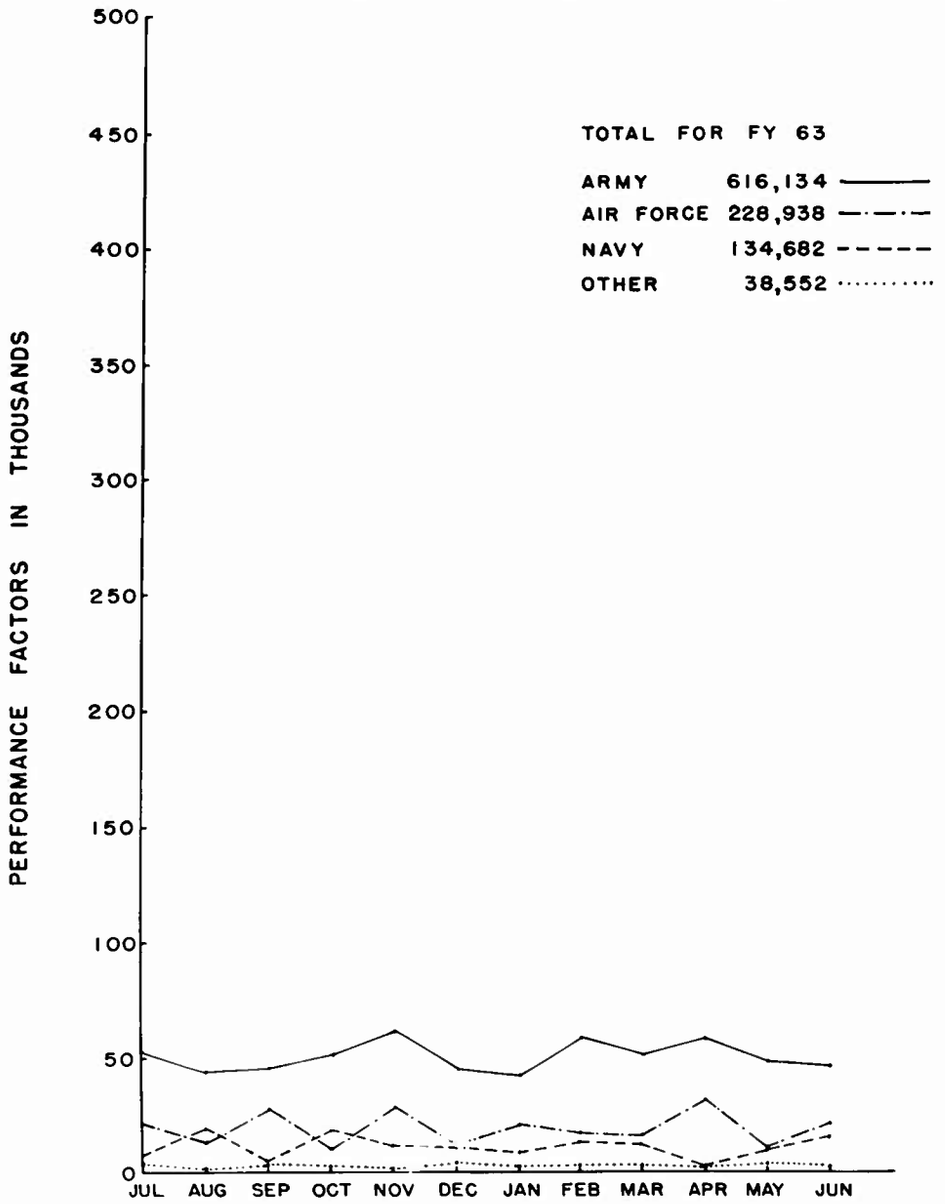
DEPARTMENT OF PATHOLOGY

PERFORMANCE FACTORS BY MONTH AND SERVICE



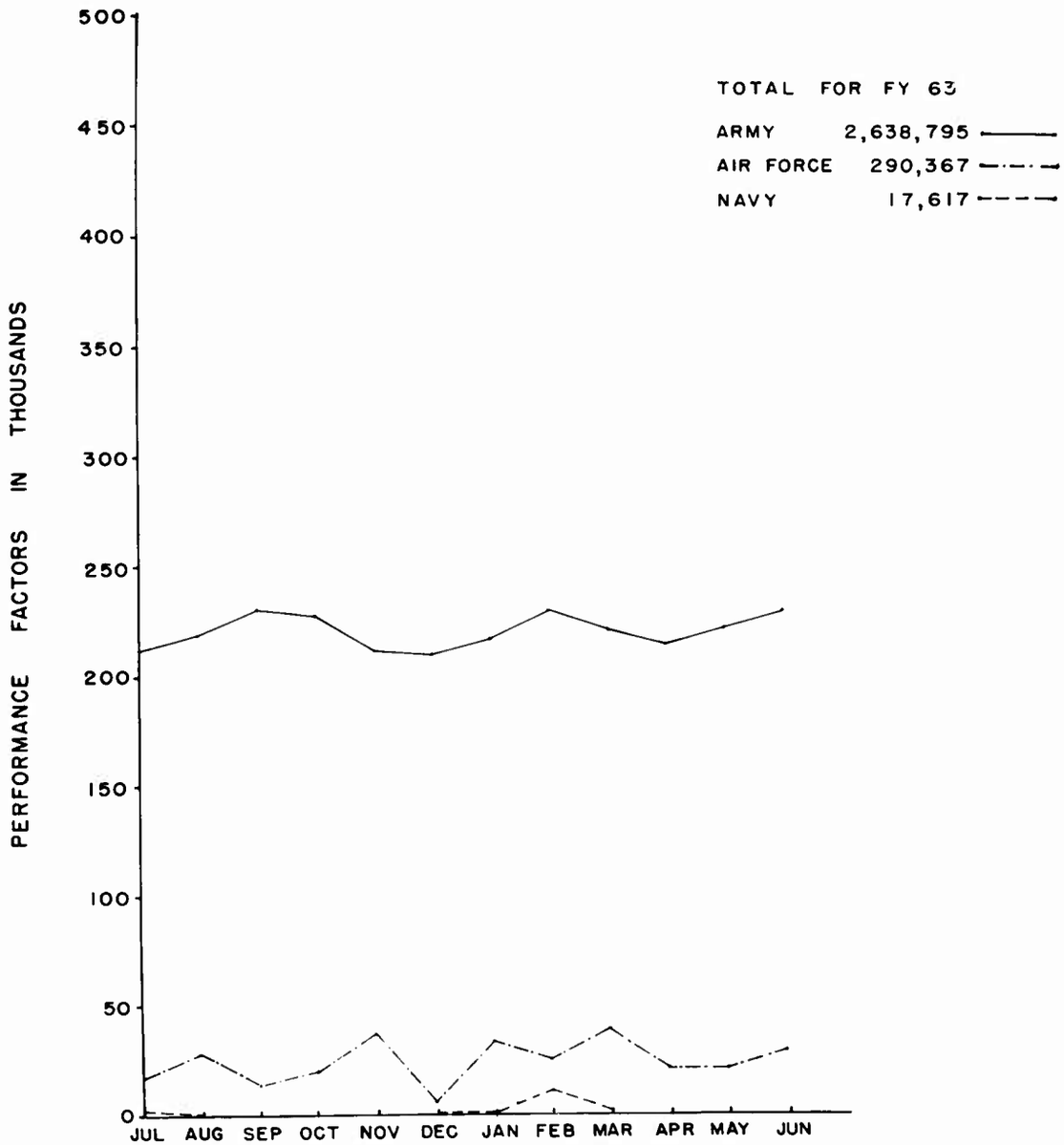
DEPARTMENT OF SEROLOGY & BLOOD BANK

PERFORMANCE FACTORS BY MONTH AND SERVICE



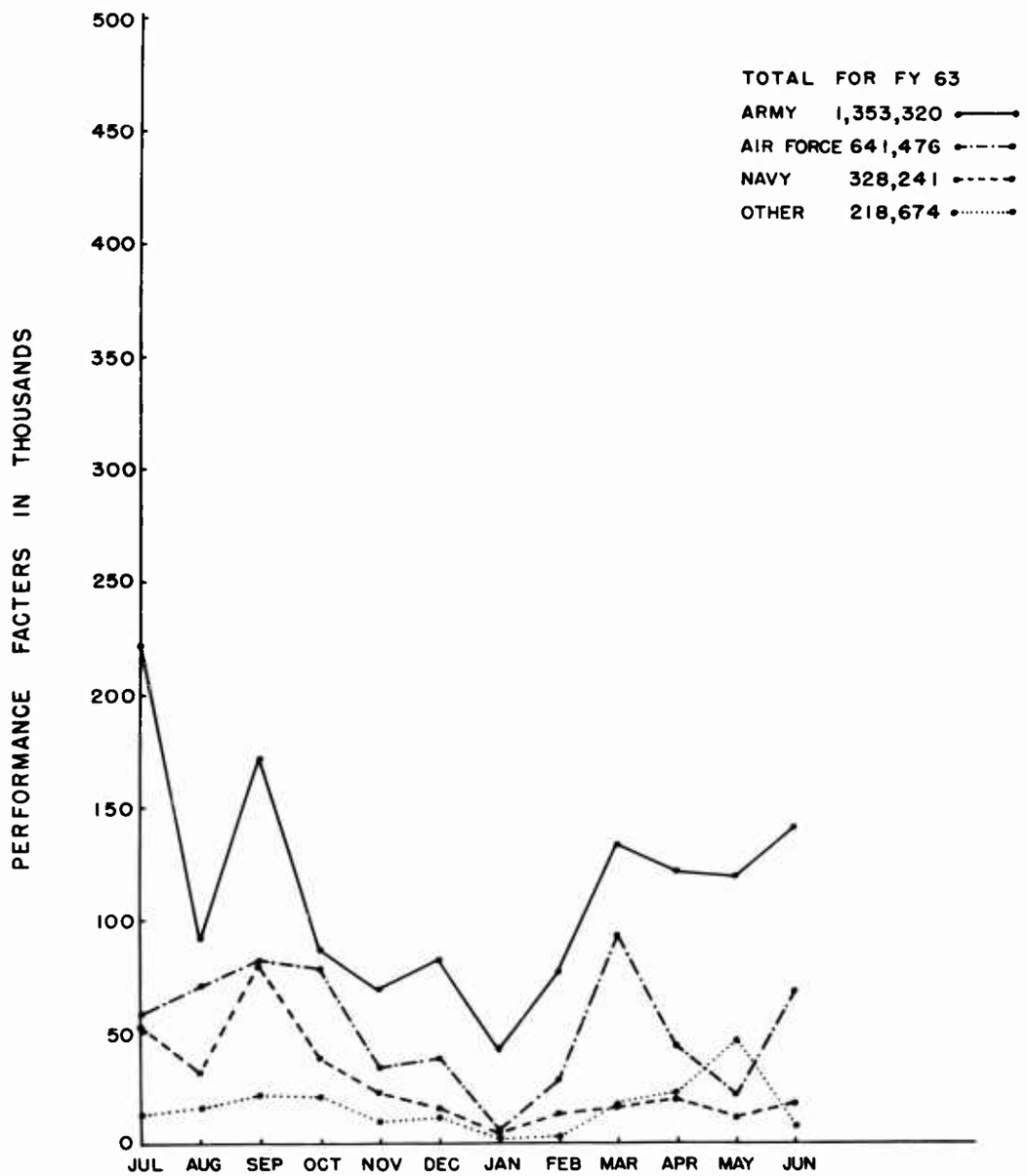
DEPARTMENT OF VETERINARY LABORATORY MEDICINE

PERFORMANCE FACTORS BY MONTH AND SERVICE



DEPARTMENT OF VIROLOGY AND RICKETTSIAL DISEASE

PERFORMANCE FACTORS BY MONTH AND SERVICE



PERSONNEL ASSIGNED

July 1962 through June 1963

ADMINISTRATION

Headquarters

Metzger, Joseph F., Lt Col, MC
Tessmer, Carl F., Col, MC, dep
Mac Nair, Donald S., Major, MC, dep
Pokras, Jacob, Major, MSC, dep
Wilson, John J., Major, MSC, trfd
Mc Intyre, Eugene J., Capt, MSC
Carriger, Barbara K., GS-7, dep
Ramsey, Ann, GS-7
Bolden, Colleen L., GS-5
Oshikata, Mitsuo, 1st Sgt (E-8)
Otis, Roy, M/Sgt (E-8), dep
Sears, Wendell S., Sp5, dep
Dawson, Napoleon, Sp5
Smoot, Llyod, Sp4, dep
Foggie, Margaret, Sp4, dep
Hender, Dorothy, Sp4, dep
Bayard, Mary E., Sp4, dep
Dykes, Bobby, PFC
Niiya, Joe, M.D., GS-11, dep
Nakajima, Hideyuki
Oshima, Ai
Kishi, Yukitsugu
Hirai, Kenichi
Saito, Kuniharu
Tashiro, Shigeo
Asami, Yozo

DEPARTMENT OF BACTERIOLOGY

Administrative

Hoefling, Adam F., Capt, MSC, dep
Mc Ilwain, Patric K., 2d Lt., MSC
Ramsey, Ann H., GS-5, trfd
Kavanaugh, Harry J., SFC, dep
Taylor, Agnes A., GS-5, dep
Pineda, Pedro, SFC

Enteric

Haga, Kyuei

Diagnostic

Petty, Thomas L., Sp5
Yaguchi, Reizo, M.D., Ph.D.
Tachibana, Yoshiko
Okada, Kiyoko, dep
Kitao, Yozo, Ph.D.
Takano, Chiyono
Della Vedova, Mario, dep

Tuberculosis

Rei, Tien S., Ph.D., M.D.
Kimoto, Kenichi T., Sp5, dep
Matsuda, Kunio

Food and Water

Tyeon, Hakun, M.D.

Anaerobics and Mycology

Kawatomari, Toshio, GS-11
Fukada, Yoko
Ito, Shigekazu, M.D.

Bio-Assay

Tanuma, Bungo
Utsugi, Motomu
Miyata, Katsuko

Media and Glassware

Narishima, Tadashi
Suzuki, Yoshiko
Kubo, Yoshiko
Saito, Kaoru, dep
Kobori, Masatsugu

Animal Room

Tsuda, Toshio

Contract Consultants

Ghoda, Akira, M.D.

DEPARTMENT OF CHEMISTRY

Administrative
- Wilson, John W., Major, MSC
- Fowlkes, Nelson J., 1st Lt, dep
- Wylie, Ellis, S/Sgt, dep
- Platt, Gerald C., Sp5
- Isobe, Choichi
- Tashiro, Shigeo

Analytical-Water
- Jung, Jurgen, Pvt, dep
- Wolff, Jimmy F., Sp4, trfd
- Kuroda, Keiji
- Nishiyama, Chizuko
- Nakamura, Akira, dep

Biophysics and Radioactivity Measurements
- Okabe, Kenzo, Ph.D.
- Yamamoto, Norihiko
- Hiraga, Yasuyuki
- Furukawa, Yoshiko

Diagnostic Clinical Chemistry
- Kinney, Roy C., Sp5
- Wicks, John W., Sp4
- Witkop, Robert M., Sp4, dep
- Yoshimura, Mari
- Yamazaki, Masaharu
- Suzuki, Kazuko, dep

Toxicology
- Kato, Yuzuru
- Komaki, Junichi

DEPARTMENT OF ENTOMOLOGY

Administrative
- Keegan, Hugh L., Lt Col, MSC
- Field, Gordon, Major, MSC, dep
- Coleman, Wilma H., GS-5
- Stamm, Richard L., Sp4, dep
- Weaver, Robert E., Jr., S/Sgt
- Poore, Charles M., PFC
- Toshioka, Seiichi, M.D.

Bionomics
- Flowers, John R., Sp5
- Matsui, Takajo
- Hatoyama, Hajime, M.D.
- Suzuki, Hiroshi
- Furuki, Motoshichi
- Goto, Setsuo
- Kunishige, Nobuo
- Narishima, Kiyoko
- Sasagawa, Shogo
- Shinonaga, Satoshi, dep
- Sugiyama, Hidezo
- Togawa, Sachiyo
- Ueno, Yoshiko
- Watanabe, Toru, dep

Personal Service Contractors
- Kano, Rokuro, M.D.
- Mizusawa, Kiyoyuki
- Nagasawa, Hatsumi, dep
- Suzuki, Mitsue, Ph.D., M.D., dep
- Tajima, Takashi

Taxonomics
- Yoshida, Yoshiko
- Daishoji, Kei
- Miyasaka, Kinuyo
- Shibata, Saburo
- Shimazoe, Akira, dep
- Ando, Takashi
- Fujisawa, Sei
- Hasunuma, Masao
- Hosokawa, Atsumi
- Misaki, Mutsuko
- Ohtawa, Shozo
- Sasaki, Yoshinori
- Sonobe, Yusaku
- Yoshigaki, Ichiro

DEPARTMENT OF MEDICAL ZOOLOGY

Administrative & Diagnostics
- Moose, John W., Major, MSC
- Ishikawa, Miyoko, GS-5
- Stroud, William H., S/Sgt
- Fleshman, Paul, Sp5
- Asakura, Soichi
- Hishiyama, Yoshio
- Miyasaka, Eikichi
- Oda, Ayako
- Kishimoto, Tadashi, dep
- Ogawa, Masanori, dep

R&D Project
- Williams, James E., GS-12
- Sadler, William O., Jr., SFC
- Miyake, Gary, Sp5
- Vaughn, Glennon C., dep
- Lin, Sung Sheng, Ph.D., M.D.
- Aoki, Katsutoshi
- Hishinuma, Yoshimasa
- Kobayashi, Hiroshi
- Nagao, Setsuko
- Tamura, Kohji
- Yamaguchi, Shohei

DEPARTMENT OF PATHOLOGY

Administrative
- Mac Nair, Donald S., Major, MC, trfd
- Stiff, David P., Captain, MC, TDY, dep
- Nishiyama, Ronald H., Captain, MC
- Schmidt, Julie K., dep
- Ramsey, Ann, GS-5, trfd
- Geddes, Betty E., GS-5
- Nawa, Kimiko

Pathology Section
- Rigger, Wilbur L., SFC,
- Mossman, James H., Sp5, dep
- Albarez, Joseph V., Sp5

Cytology
- Wakabayashi, Kikuko

Special Stains
- Mitsuhashi, Toshimachi

Tissue Processing and Stains
- Suzuki, Yasuko
- Murata, Ieji

Electron Microscopy
- Hatano, Manabu

Medical Illustration
- Laxon, James F., Sp5, dep
- Berry, Gary L., Sp4
- Go, Tenjun
- Ochiai, Atsuo
- Tuckish, John W., Sp5, dep

DEPARTMENT OF SEROLOGY AND BLOOD BANK

Administrative
- Pokras, Jacob, Major, MSC, dep
- Collins, William S., II, Captain, MSC
- Engbrecht, Dale, SFC
- Osawa, Tokuko

Blood Bank
- Platt, Gerald C., Sp5, trfd
- Wicks, John W., PFC, trfd
- Palenske, Carlton L., Sp5
- Matsunuma, Kuni

Serology
- Larson, Lars B., SFC, dep
- Albritton, Arthur, SFC
- Muto, Toshio
- Liu, Keeup
- Suzuki, Shiro
- Takahashi, Mitsuko
- Katsumata, Shizuyo

DEPARTMENT OF VETERINARY LABORATORY MEDICINE

Administrative
- Rodman, Ivan F., Jr., Captain, VC
- Trevino, Gilberto S., Captain, VC, dep
- Banta, Carrol L., SFC
- Nakamura, Chieko

Food Chemistry
- Albritton, Arthur, SFC, trfd
- Bishop, Edgar M., Sp5
- Palenske, Carrol L., Sp5, trfd
- Hisamatsu, Ichibei
- Mannen, Masahiro

Food Bacteriology
- Wolff, Jimmy F., PFC, trfd

Infectious Diseases
- Furusho, Yutaka

Diagnostic Animal Supply
- Namiki, Seiji
- Shimazaki, Chiaki
- Hirayama, Takashi
- Kojima, Shigeji

DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES

Administrative
- Gershon, Richard K., M.D., GS-12, dep
- Morgan, Jean F., GS-5
- Swanton, Lyle G., SFC
- Iwasaki, Naoko

Research
- Chapman, Merdith E., S/Sgt
- Coleman, Wilma H., GS-3, trfd
- Fuji, Hiroshi, M. D., dep
- Fujinaga, Chisato, dep
- Fujinaga, Kei, dep
- Fujisaki, Yukiro, M. D.
- Kondo, Kazunari
- Sato, Yukiko
- Shimada, Tomiko
- Taguchi, Fumiaki, dep

Serology
- Bussa, William, Sp5, dep
- Iida, Shoichi
- Kogure, Ryuko
- Mimura, Saburo

Cell Culture
- Aiki, Toshio, dep
- Tanabe, Masaji, dep

Equipment Preparation
- Ichikawa, Masatoshi
- Oinuma, Masao
- Yokota, Nagamitsu

Animal Care
- Murano, Akio
- Okada, Kojiro, dep
- Okamoto, Ihei

UNCLASSIFIED