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STUDIES OF TAIWAN LEECHES

III. Further Tests of Repellents Against Aquatic, Blood-Sucking Leeches¹

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ABSTRACT

Laboratory and field tests were conducted to determine effectiveness of eleven repellent formulations applied to the skin, and two formulations designed for clothing application in repelling blood-sucking aquatic leeches of genus Hirudo. In laboratory tests, a formulation consisting of equal parts of DEET, silicone, and a combination of eucerite and petrolatum gave complete protection against leech attachment after treated hands had been immersed in gently running water for a total of 30 minutes. After an additional 10 minute immersion, only one of 20 leeches present attached to the treated hand. Another formulation consisting of equal parts of M-2020, silicone, and anhydrous wool fat gave complete protection for a treated hand which had been immersed for only 10 minutes, but gave complete protection for the other treated hand of the same subject until a total of 80 minutes immersion had been reached. A formulation composed of M-2020-45%, and anhydrous wool fat-55% applied to the skin gave complete protection from leech attachment to one subject for 100 minutes, and 66-70 minutes complete protection to another subject protected with the same repellent. Another formulation consisting of equal parts DEET -50% and a combination of eucerite and petrolatum was second best. This formulation gave complete protection against leech attachment for 76-70 minutes to one subject, and 56-60 minutes to another. Loosely woven, untreated, cotton socks gave complete protection against leech attachment on the feet of subjects exposed for 30 minutes in a leechinfested ditch. Subjects wearing untreated, Army fatigue trousers and those wearing trousers treated with either M-1960 or benzylbenzoate received no leech bites during 120 minutes exposure in leech-infested rice paddies.

In field tests at Cha'o Chow, Pingtung, Taiwan, it was found that a formulation composed of DEET-45% and anhydrous wool fat-55%, and one consisting of DEET-40% and a combination of eucerite and petrolatum were equally effective, *i.e.* each group of 10 subjects suffered a mean leech attachment rate of only 0.2 after 30 minutes exposure in a leech-infested ditch. Pre-test, two minutes exposure

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2 Respectively: Lieutenant Colonel, MSC, U. S. Army; S/Sgt (E-6), U. S. Army; Sp5 (E-5), U. S. Army and 2nd Lieutenant, MSC, U. S. Army. attachment rates were 1.8 and 4.3 respectively. In a confirmatory test with DEET and combination of eucerite and petrolatum, the mean attachment rate for a group of five people after 40 minutes exposure was again 0.2. The pre-test, two minute exposure period mean attachment rate for this group was 5.0.

During April, 1964 field tests of effectiveness of four insect repellent formulations against aquatic, blood-sucking leeches were conducted by two of the authors (HLK and REW) at Cha'o Chow. Pingtung, Taiwan. Results of these tests were subsequently reported as the second in a series of studies on Taiwan leeches (1). These results, and additional information obtained during laboratory tests of repellents in Japan, and field tests in Korea during May 1964 by two of us (REW and PF) served as the basis for design of a final series of tests, carried out at Cha'o Chow during August 1964. This paper is written to present results of the preliminary laboratory tests and Korean field tests, as well as of the August field tests at Cha'o Chow.

MATERIALS AND METHODS (General)

Initially, eleven repellent formulations for skin application were prepared for evaluation. The active repellent in six of these was a military issue item, insect repellent, personal application (75% diethyltoluamide). The remaining formulations each contained a former item of military issue: M-2020, consisting of 30% each of dimethyl phthalate; 2ethyl-1,-3-hexanediol; and dimethyl carbate. These repellents were prepared for testing in various combinations with anhydrous wool fat; a combination of eucerite and petrolatum; a silicone; and a hydrophilic ointment. Formulations were:

- 1. DEET, silicone, hydrophilic ointment..each 33 1/3%
- 2. DEET, silicone, anhydrous wool fat. each 33 1/3%
- M-2020, silicone, combination of eucerite and petrolatum..each 33 1/3%

- 4. M-2020, silicone, hydrophilic ointment. each 33 1/3%
- 5. DEET and silicone..each 50%
- 6. M-2020 and silicone..each 50%
- DEET, silicone, and combination of eucerite and petrolatum..each 33 1/3%
- M-2020, silicone, anhydrous wool fat..each 33 1/3%
- 9. DEET..40%, combination of eucerite and petrolatum..60%
- 10. M-2020..45%, anhydrous wool fat ...55%
- 11. DEET..45%, anhydrous wool fat ...55%

Separate evaluations of effectiveness of anhydrous wool fat, the combination of eucerite and petrolatum, and the silicone used in repellent formulations were also conducted. All formulations were prepared in the pharmacy at least one week before they were used in the field.

Two repellent formulations, both military issue items designed for application to clothing, were tested. These were:

- Insect repellent, clothing application (Formula M-1960) (Active ingredients in this formula are 30% each of N-butylacetanilide; benzylbenzoate, and 2-butyl-2ethyl,3-propanediol).
- 2. Insect repellent, clothing application (90% benzylbenzoate)

Clothing used in field tests consisted of U.S. Army fatigue trousers (cotton, sateen) and loosely woven cotton socks purchased on the market at Cha'o Chow, Pingtung, Taiwan.

The remaining details concerning materials and methods utilized in the laboratory repellent tests, the May field tests in Korea, and the final tests on Taiwan are to a considerable degree unique in each case. For this reason, information concerning each of these operations is described separately.

MATERIALS AND METHODS UTILIZED IN LABORATORY TESTS OF EFFECTIVE-NESS OF TWO REPELLENT FORMULATIONS

On 20 April 1964 laboratory tests were conducted to determine effectiveness of two repellent formulations against blood-sucking leeches, *Hirudo* sp., from Taiwan. Formulations tested were:

- a. DEET, silicone, and a combination of eucerite and petrolatum..each 33 1/3%
- b. M-2020, silicone, and anhydrous wool fat.each 33 1/3%

One subject tested each repellent. In carrying out the test one and one-half ml of repellent mixture was rubbed onto the skin of both hands. After a waiting period of five minutes, one hand was immersed in a tank 115/8'' deep and 115/8''in diameter, into which water was flowing at a rate of two gallons per minute. Water temperature was 32 C.

The immersed hand was held motionless for a period of two minutes and was then removed from the water for a one minute period. At the end of a minute the hand was again placed in the water, and the sequence repeated. Five immersions of this type were made before the hand was exposed to 20 leeches in an animal jar 8'' high and 81/4'' in diameter, which contained water to a depth of two inches. If leeches crawled on the hand and attached, the other hand (which had not been previously immersed in flowing water) was exposed after each immersion period for a two minute exposure. If no leeches attached to the test hand, another series of five immersions commenced, and the series was continued. After attachment occurred with the first hand, the other hand was immersed a number of series only one less than the first hand, and then exposed after each immersion until attachment occurred.

MATERIALS AND METHODS UTILIZED IN FIELD TESTS OF LEECH REPELLENTS AT KUMPO, NAM-MYUN, KYUNG-GI PROVINCE, KOREA

On 28 and 29 May 1964 the eleven repellent formulations for application on the skin, and the two formulations for treatment of clothing were tested for effectiveness in the field at Kumpo, Korea, against aquatic leeches of genus Hirudo, probably H. nipponia. Tests were conducted in a rice paddy, and in irrigation reservoirs in paddies. Each of these reservoirs was about 20 feet long, 15 feet wide, and from three to five feet deep. There was some aquatic vegetation, including rushes along the reservoir margins. The paddy in which tests were conducted was about 55 yards long and 35 yards wide. Water depth varied from mid-calf to knee level. The soil in the area was very fine, slippery clay. Water temperature varied only slightly, from 22-24 C. Water pH varied from 6.0-6.5. Test subjects were three U.S. military personnel, and nine Korean men.

Tests of effectiveness of repellents applied to the skin were conducted in the following manner: One subject was assigned to each repellent formulation and given the same numerical designation as the repellent. Subjects removed foot gear, rolled trouser legs to the mid-thigh level, and themselves applied the repellent formulations to all exposed skin of the feet and legs. Exposure to leeches was effected by stationing subjects on a reservoir bank at double-arms' length interval. with legs dangling in the water. Upon signal all subjects immersed their legs in the water and kicked vigorously for one minute, after which they remained motionless for four minutes. This five minute combination constituted one exposure At the end of each exposure period. period subjects stood on the bank while leeches were counted by monitors. Leeches counted were recorded as being either present or attached.

Two tests were conducted. The first, in which all eleven formulations were tested, consisted of a total of 10 fiveminute exposure periods, with a fiveminute break between the fifth and sixth periods. The four most effective formulations, judged by duration of complete protection from leech attachment were then re-tested in a series of 20 five-minute exposure periods. Upon completion of a test, subjects washed legs with soap and water and rubbed down with isopropyl alcohol.

Tests of effectiveness of untreated U.S. Army fatigue trousers and trousers treated with M-1960 or benzylbenzoate in protecting personnel from leech attachments were carried out by nine subjects in the rice paddy previously described.

Three of these men wore fatigue trousers impregnated with M-1960, three wore trousers impregnated with benzylbenzoate, and three wore untreated trousers. Trousers were tucked into sock tops (no shoes were worn) and the union of sock and trouser was taped at the ankle. In carrying out the test, subjects, at double-arms' length interval, entered the water simultaneously on command, walked for one minute, then stood quietly for four minutes. Upon signal they again started walking for a one minute period, followed by a four minute stationary period. Upon completing this cycle three times (for a total of 15 minutes exposure, counted as one exposure period), they ascended the bank and leeches were counted. After two exposure periods (30 minutes) trousers were dropped and skin examined for leeches or leech bites inside clothing. Following this the test procedures were repeated until a total of eight exposure periods, or 120 minutes exposure time had been reached.

Prior to each exposure period, an unprotected subject agitated the water to insure that blood-sucking leeches were present. In every case leeches appeared and attacked within 10-30 seconds.

Treated and untreated trousers were

chosen by subjects in a random manner. Until the end of the first two exposure periods neither subjects nor monitors knew which subjects were wearing treated garments.

FIELD TESTS OF AQUATIC LEECH REPEL-LENTS AT CHA'O CHOW, PINGTUNG, TAIWAN, AUGUST 1964

Initially, four repellent formulations which had given good results in screening tests in Korea were evaluated. The active ingredient in each formulation was the military item, insect repellent, personal application (75% diethyltoluamide), These formulations were:

- 1. DEET....40% combination of eucerite and petrolatum....60%
- 2. DEET....45% anhydrous wool fat....55%
- 3. DEET, silicone, and combination
- of eucerite and petrolatum.... each 33 1/3%
- 4. DEET, silicone, and anhydrous wool fat...each 33 1/3%

Separate tests were run to determine effectiveness of anhydrous wool fat alone, and of the combination of eucerite and petrolatum alone in repelling leeches.

M-2020 was not utilized in Taiwan field tests since this formulation was discontinued as a military item of issue due in part to its lack of acceptability to the troops.

Thirty-three residents of the Cha'o Chow area participated in field tests conducted daily between 5-8 August 1964. Thirty of these people served as test subjects. The remaining three acted as monitors, and applied repellents to legs of test participants. An additional 20 individuals were hired on a part-time basis for control tests to determine leech biting rates immediately following repellent tests. There was a considerable size variation among participants.

All tests were conducted in irrigation ditches in the general area utilized for

the April 1964 tests described in Study No. 2 of this series (1). Ditches were selected as test sites after it was determined that adequate populations of bloodsucking leeches, Hirudo sp., were present. Width of the ditches utilized varied from about 21/4 feet to 10 feet. Water depth in these ditches varied from less than one foot to more than two feet. It was difficult to accurately determine "test" water depth, as subjects often sunk several inches in Density of aquatic vegetabottom mud. tion and rate of water flow varied in different ditch sections. Although both water level and rate of flow showed some daily variation, this produced no noticeable effect on leech populations.

Basic testing procedures closely followed those described in Study No. 2 of this series (1). Each person hired as a test subject was assigned a number which was retained by the individual throughout the four day test period. Before tests, subjects removed shoes and socks, and either removed trousers, or rolled trouser legs up above the mid-thigh level. Test subjects were directed to specific points along the ditch margins by monitors. In all tests, subjects, upon signal, stepped into the water, and either "walked-in-place" or gently kicked the feet for a period of one At the end of the first minute, minute. a signal was given and all subjects remained motionless until they were directed to step out on the bank, where they stood motionless until counts of attached leeches has been made by monitors. Applications of repellents were made by monitors. Because of variation in size of participants, no effort was made to apply an equal amount of the formulation being tested to each subject. The objective was to apply the formulation to the feet and legs of the participants until all skin surface up to the mid-thigh level was visibly covered. Subjects were cautioned not to step into areas of the ditch where water would reach unprotected skin above the mid-thigh. Where water was particularly deep, subjects were allowed to sit on the bank and dangle the legs in the water. At the end of each exposure period during a test, attached leeches were counted and removed, taking care that repellent was not wiped from the skin in the process. Because of this possibility, even freely bleeding attachment wounds were not treated until completion of the entire test.

As a result of experience gained during the April 1964 field tests in the Cha'o Chow area, 5 and 6 August were devoted to careful instruction of participants and monitors in test procedures; location of good test sites; and preliminary tests necessary to determine effectiveness and practicality of procedures.

One such preliminary test was set up with two objectives:

1. To determine how many of 30 persons exposed without repellent for two minutes in a leech-infested ditch would suffer leech attachments.

2. To determine duration of complete protection (*i.e.*, no attachments) afforded by applications of each of three formulations containing diethyltoluamide, and each of the three inert ointment bases used in preparing repellent formulations. In carrying out this test, participants were divided into six groups of five persons each. Each group tested effectiveness of a single formulation. During these tests each subject wore looselv woven cotton socks purchased on the local market. Sock tops were rolled to to ankle level.

The final test procedure, designed after completion of preliminary tests, consisted of three phases:

1. A preliminary two minute exposure period, without repellents, to insure that the leech attachment rate at the time was adequate for testing purposes.

2. The actual test phase, which was divided into three exposure periods of at least 10 minutes each. During this phase, test participants were divided into groups of 10 each. Participants in two of these groups were given applications of formulations containing diethyltoluamide. Members of the third group received applications of anhydrous lanolin only. This placebo formulation was of the same odor, appearance, and consistency as the actual repellent formulation. Test subjects were not told of the nature of any of the formulations.

3. A "post-test" exposure period to determine whether the reduced attachment rates during the actual test were due to effectiveness of materials used or to other factors such as a reduction in the leech population in the ditch as a result of preliminary tests. Subjects hired for this exposure period had not participated in other phases of the test.

All tests were conducted during the daytime. Water temperature and pH were determined immediately prior to each test.

Effectiveness of a formulation as a

repellent was judged by duration of complete protection given exposed persons protected by repellents, and by comparing attachment rates of protected and unprotected individuals.

RESULTS AND DISCUSSION

Results of laboratory and field tests with repellents are presented in TABLES I-VII.

Since the number of ways in which repellents against aquatic leeches can be tested is quite limited, techniques employed in the laboratory test, and in the Korean and Taiwan field tests, to a considerable extent duplicated methods described by Wilson (2); Harrison, Audy and Traub (3); and Harrison and Audy (4). Differences which may be of significance include the amount of repellent formulation applied to each subject, the criteria for judging repellency, and the

TABLE I

Results of laboratory tests of effectiveness of two repellent formulations against blood-sucking aquatic leeches

А.	Protection afforded subjects whose hands were treated with a formulation consisting of
	equal parts of DEET, silicone, and a combination of eucerite and petrolatum:
	Leeches present or attached after a two minute exposure period following:
	1st series of five, two minute washings $\dots 3P^*$
	2nd series of five, two minute washings
	3rd series of five, two minute washings4P
	4th series of five, two minute washings $\dots 1A^*$
В.	Protection afforded subjects whose hands were treated with a formulation consisting of
	equal parts of M-2020, silicone, and anhydrous wool fat:
	(1st hand)
	Leeches present or attached after a two minute exposure period following:
	1st series of five, two minute washings2P
	2nd series of five, two minute washings5P, 1A
	(2nd hand)
	Leeches present or attached after a two minute exposure period following:
	1st series of five, two minute washings2P
	6th immersion3P
	7th immersion
	8th immersion
*Sym	bols: P-Present.

A-Attached.

nature of control tests in the field.

In tests conducted by 406th Medical Laboratory personnel at Kumpo, Korea and Cha'o Chow, Taiwan no attempt was made to apply an equal amount of repellent to each test subject. Subjects varied considerably in size, and to a certain extent in amount of hair on legs. Because of this, it was decided to apply repellent until all exposed skin surface was visibly covered. This practice probably assured a fairly close approach to the rate of application which would be expected of troops in the field in leech-infested areas.

In field tests in Borneo against aquatic leeches, Harrison, Audy and Traub (3) found that both treated and untreated clothing gave excellent protection when trouser bottoms were tucked into sock tops. Similar results were obtained by Keegan and Weaver (1), and field tests in Korea (TABLE IV). In addition it was found that at Cha'o Chow, Taiwan, untreated loosely woven, cotton socks gave complete protection against leech attachments on feet during 30 minutes exposure. In contrast, Harrison, Audy and Traub (3) reported that thick, knitted, woolen socks of standard Army issue were of little value against aquatic leeches, although they were effective against land leeches.

Field tests effectiveness of skin applications of repellents against aquatic leeches have usually involved treatment of one or both legs of a subject, and comparison of numbers of leeches present after one or more exposure periods with those present on legs of unprotected subjects. (1, 3, 4) In instances where a field

TABLE II

Effectiveness of skin applications of eleven repellent formulations against aquatic blood-sucking leeches at Kumpo, Korea*

Subjects-Formulations**	Duration of complete protection (minutes)	present an	total leeches ad attached utes exposure
1. DEET, silicone, hydrophilic ointment —each 331/3%	6-10***	$3P^{\dagger}$	9 <i>A</i>
2. DEET, silicone, anhydrous wool fat each 33 ¹ / ₃ %	6-10	1 <i>P</i>	5A
 M-2020, silicone, combination of eucerite and petrolatumeach 331/3% 	26-30	7P	4A
 M-2020, silicone, hydrophilic ointment —each 33⅓% 	16-20	1P	1A
5. DEET and silicone—each 50%	1-5	11 P	11A
6. M-2020 and silicone—each 50%	6-10	8P	22A
7. DEET, silicone, and combination of eucerite and petrolatum—each 331/3%	26-30	11P	3A
 M-2020, silicone, and combination of eucerite and petrolatⁿm—each 33¹/₃% 	36-40	1P	3A
9. DEET and combination of eucerite and petrolatum—each 50%	50	0P	0A
10. M-2020-45%, anhydrous wool fat-55%	46-50	1 <i>P</i>	1A
11. DEET-45%, anhydrous wool fat-55%	36-40	2P	1A

* Repellent applied to bare feet and legs up to mid-thigh level.

** Each formulation was tested on one subject.

^{***} Duration of complete protection is the interval between commencement of exposure and the first leech attachment. Subjects were exposed in successive five minute periods. At the end of each period subjects were examined for leech attachments.

⁺ In leech counts the letter A stands for leeches attached and feeding. The letter P stands for leeches present on the skin but not attached.

Table I	Π
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Effectiveness of skin applications of "Four Best" repellent formulations against aquatic blood-sucking leeches at Kumpo, Korea*

		Duration of complete protection (minutes)	Cumulative present and after 50 minu	d attached
Formulation no. 4 M-2020, silicone, and hydro ointment—each 33½%	ophilic			
Subjects:	a	11-16**	1 P***	15 P
	b	31-35	3P	7A
			Cumulative to present and after 100 min	l attached
Formulation no. 9 DEET-50%, combination of and petrolatum-50%	of eucerite			
Subjects:	a	76-80	0 <i>P</i>	2A
	b	56-60	4 P	5A
Formulation no. 10 M-2020-45% anhydrous wool fat-55%				
Subjects:	a	100-?	2 P	0A
	Ъ	66-70	2 P	4A
Formulation no. 11 DEET-45% anhydrous wool fat-55%			·	
Subjects:	a	56-60	3 P	4A
	b	51-55	0 P	5A

* Repellents applied to bare feet and legs up to the mid-thigh level.

** Duration of complete protection time is the interval between commencement of exposure and the first leech attachment. Subjects were exposed in successive five minute periods. At the end of each period subjects were examined for leech attachments.

*** In leech counts the letter A stands for leeches attached but not feeding. The letter P stands for leeches present on the skin but not attached.

test of leech repellents is preceded by a control test of non-protected individuals, it is possible that low 'counts during the test proper may be due to depletion of the leech population by the initial exposure during the control test. Because of this it seems desirable that an additional control test be conducted along with the repellency test, or that the latter be followed by an additional exposure of unprotected individuals. Harrison, Audy, and Traub (3) found that none of several ointments tested in Borneo was particular-, ly effective and that no complete protection was afforded. Leeches were present on both treated and untreated legs during the first half hour of exposure, and during the succeeding 45 minutes all apparent effect was lost. Our own results in Korea and Taiwan were more encouraging, as indicated by data shown in TABLES II, III, V, VI and VII.

Harrison, Audy and Traub (3) and Audy and Harrison (4) judged effectiveness of repellent formulations by comparing numbers of leeches and bites on protected and unprotected subjects after exposure periods of varying length. It seems probable that a more accurate method for determining repellency would be that described by Keegan and Weaver (1) of distinguishing leeches actually

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TABLE IV

Results of field tests to determine effectiveness of untreated U.S. Army fatigue trousers, and trousers treated with either M-1960 or benzylbenzoate in preventing attachment of blood-sucking leeches*

Subject no.	Trousers treated with	Leeches present or attached during each of eight consecutive 15 minute exposure periods						Total		
		1st	2nd	3rd	4th	5th	6th	7th	8th	
1	M-1960	1P]				1P
2	Benzylbenzoate	1 P								1P
3	M-1960	1P	1P						,	2P
4	Benzylbenzoate									$0\boldsymbol{P}$
5	Untreated	1P	2P	1P		1P		1 P		6P
6	Benzylbenzoate		1 P							1P
7	M-1960	1P								1P
. 8	Untreated	3P			1P		1P			5P
9	Untreated	1P		1P	1 P				2 P	$\mathbb{E}P$

* Each subject had trousers tucked into tops of cotton socks, and junction of sock and trouser taped. Subjects wore no shoes.

TABLE V

Relative protection against aquatic leech attachments afforded by skin applications of three diethyltoluamide repellent formulations and three inert vehicle, components of repellent formulations*

Groups (5 subjects each)	Mean protection time (minutes) before first attachment			
I. Protected by: DEET-40% Combination of eucerite and petrolatum-60%	35			
I. Protected by: DEET-45% Anhydrous wool fat-55%	29.6			
II. Protected by: DEET-33¼% Silicone-33¼% Combination of eucerite and petrolatum-33⅓%	12			
IV. No repellent Combination of eucerite and petrolatum only	25.8			
V. No repellent Silicone—50% Combination of eucerite and petrolatum—50%	6.2			
VI. No repellent Anhydrous wool fat only	21.6			

* Repellent applied to feet and legs of test subjects up to the mid-thigh level.

attached separately from those present on the skin but not feeding. It was frequently observed that leeches would "explore" on protected and unprotected skin but would eventually drop off without feeding. When subjects left the water TABLE VI

Effectiveness of skin applications of anhydrous wool fat and of two repellent formulations containing diethyltoluamide in preventing aquatic leech attachments

	Attached leeches on bare feet and legs after:				
	2 minute pre-test exposure period	lst 10 minute test exposure period	2nd 10 minute test exposure period		
Group I. No repellent Anhydrous wool fat only				•	
Subject No. 1 2 3 4 5 6	2 5 1 2 2 1	0 2 0 2 0 0	0 0 0 0 0 0	0 0 0 0 0 0	
7 8 9 10	3 4 2 5	0 0 0 0	0 2 0 2	0 0 0 0	
Total Mean	27 2.7	$\begin{array}{c} 4\\ 0.4 \end{array}$	4 0.4	0 0	
		al attachments f an attachment ra		ites): 8 0.8	
Group II. Protected by: DEET-40%, Combination of eucerite and petrolatum-60%					
Subject No. 11 12 13 14 15 16 17 18 19 20	5 3 4 4 14 3 2 3 2	$ \begin{array}{c} 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$	$ \begin{array}{c} 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$	0 0 0 0 0 0 0 0 0 0	
Total Mean	43 4.3	1 0.1	1 0.1	0 0	
		al attachments fo n attachment ra	•	tes): 2 0.2	
Group Ⅲ. Protected by: DEET-45%, Anhydrous wool fat-55%					
Subject No. 21 22 23 24 25 26 27 28 29 30	6 2 1 2 3 0 0 2 1 1	0 0 0 0 0 0 0 0 0 0	0 0 0 1 1 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0	
Total Mean	18 1.8	0 0	2 0.2	0 0	
		al attachments fo an attachment ra		tes): 2 0.2	

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Post-test exposure period. No repellents of any kind applied.	Leeches attached after 2 minute exposure period			
Subject No. 1	3			
2	3			
. 3	4			
4	· 1			
5	11			
6	3			
7	1			
8	1			
Total	27			
Mean	3.37			

some of these exploring leeches often remained on the skin. This phenomenon is illustrated by results shown in TABLES II and III. Another factor which should be taken into consideration in judging effectiveness of repellency is the apparent occurrence of "pockets" of leeches at close intervals in the same ditch. This distribution pattern has given some odd results during field tests in several areas. For example, in one test reported by Harrison, Audy and Traub (3) 12 leeches were counted on the right leg of an unprotected test subject, while 23 leeches were present on the left leg of the same individual. Similarly, in tests at Taiwan there were often striking differences in numbers of leech attachments on unprotected test subjects standing close to one another in a ditch. This disparity is examplified in test results shown in TABLE VI.

Unfortunately, the question as to whether any repellent formulation in ointment form will be acceptable to troops in the field is as yet undetermind. The lack

Results of a test to confirm effectiveness of a repellent formulation composed of 40% diethyltoluamide and 60% of a combination of eucerite and petrolatum in preventing attachment of aquatic leeches*

	Attach	Attached leeches on bare feet and legs after						
Subject no.	2 minute pre-test exposure period	lst test exposure (10 minutes)	2nd test exposure (20 minutes)	3rd test exposure (10 minutes)				
1	10	1	0	0				
2	8	• 0	0	0				
3	3	0	0	0				
4	2	0	0	. 0				
5	2	0	0	0				
Total	25	1	0	0				
Mean	5	0.2	0	0				
······································	Tota	Total attachments for test (40 minutes): 1						

Mean attachment rate for test:

0.2

Post-test exposure period.	Leeches attached to bare feet** and legs after			
No repellents of any kind applied.	2 minute exposure period			
Subject no. 1	0			
2	0			
3	5			
4	0			
5	0			
6	2			
7	2			
8	4			
9	2			
10	4			
11	0			
12	0			
Total	19			
Mean	1.6			

* Repellent applied to legs of subjects up to the mid-thigh level.

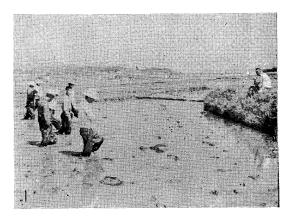
** Feet were protected by loosely woven cotton socks.

of odor of DEET and of the vehicles used in the repellent formulations tested in Taiwan may be offset by the rather "messy" physical nature of the preparations. It seems likely, however, that any aesthetic objections to use of the repellent formulations might be overcome by the sight of the many freely bleeding lesions suffered by unprotected individuals after only a few minutes in leech-infested water. Any person wading through such areas, or forced to swim, crawl, or lie motionless for any length of time in water where blood-sucking leeches are numerous would probably be eager to use any kind of repellent.

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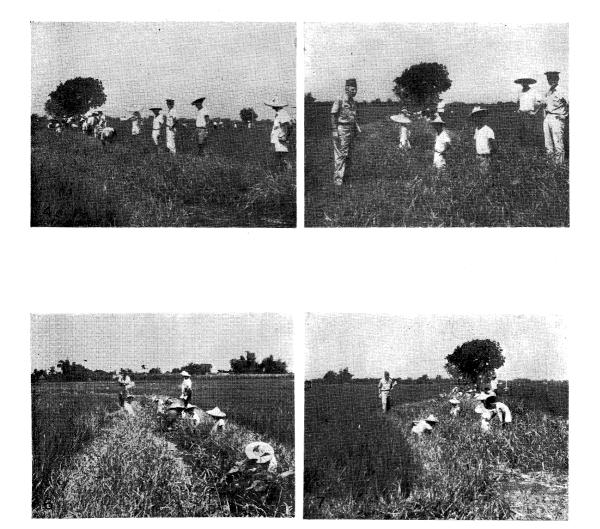
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Figs. 1-3. Photographs taken during field tests of leech repellents at Kumpo, Korea.



Figs. 4-7. Photographs taken at Cha'o Chow, Pingtung, Taiwan, during field tests of leech repellents.