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SMITH, C.N. ET AL 1 OF 1

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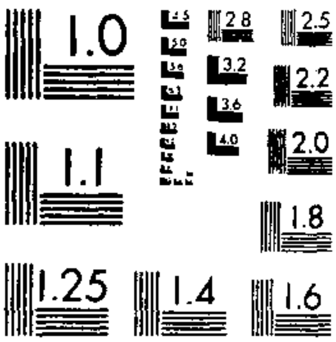

MICROCOPY RESOLUTION TEST CHART
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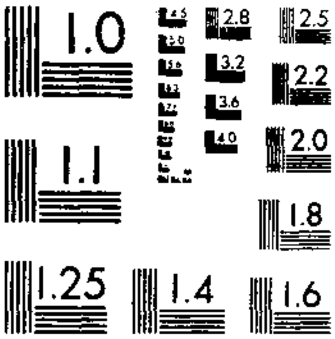

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Factors Affecting the Protection Period of Mosquito Repellents

Technical Bulletin No. 1285

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

Contents

Washington, D.C. Issued July 1963

Factors Affecting the Protection Period of Mosquito Repellents

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Since 1942 thousands of compounds have been screened and evaluated as mosquito repellents at Orlando, Fla. These studies have been summarized by King (*1954*) and Gilbert et al. (*1957*).[2] In most of this work the criterion of effectiveness has been the protection period, or length of time a liberal application of the repellent has prevented mosquitoes from biting through treated skin or clothing. This criterion has also been used in most of the insect-repellent studies by Granett (*1940*), Pijoan et al. (*1945*), Pijoan (*1947*), and Kasman et al. (*1953*). Occasionally effectiveness has been judged by the minimum concentration required to prevent biting at the time of application, or the initial repellency of a very small dosage. This second criterion was used in studies by Altman and Smith (*1955*), Bar-Zeev and Smith (*1959*), Gilbert et al. (*1957*), and Gouck et al. (*1957*). The protection period usually varies greatly, not only between repellents but also between different individuals with the same repellent, different populations of mosquitoes, and different environmental conditions.

In 1957 studies were undertaken at Orlando to determine the factors that affect the length of the protection period, with particular emphasis on the manner in which the repellent is lost from the treated surface.

The protection period conveyed by any given dosage of repellent obviously depends on (1) the minimum effective dosage, hereafter designated the MED, which is the minimum amount per unit of surface required to protect against the given population of insects, and (2) the rate at which the applied dosage is depleted to the level of the MED, that is, the rate of loss. The MED may be set at any level, such as 99-percent protection, 50-percent protection, one confirmed bite, or five bites in 3 minutes. However, at any given level it will presumably be affected by factors other than those inherent in the repellent, such as (1) the avidity of the insects and (2) the desirability of the host. Such environmental conditions as temperature and relative humidity may be expected to exert the greater part of any effect they may have on the MED through one of these two factors, although high temperature conceivably could increase the effectiveness of a repellent by increasing the rate of volatilization and thus affect the MED.

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[1] The authors are indebted to A. W. Lindquist for many valuable suggestions during these studies and to Nelson Smith, David W. Meifert, Richard L. Fye, and Rouselle A. Sutton of this Division for assistance in conducting the experiments.

[2] The year in italics after the author's name is the key to the reference in Literature Cited, p. 36.

The rate of loss has been presumed to depend principally on actual physical loss, which may be due to (1) abrasion, (2) evaporation, and (3) absorption. Destruction of the repellent on the skin has also been postulated as a possible mode of loss by Kasman et al. (*1953*). At the beginning of these studies it also appeared possible that the repellent might lose effectiveness on the skin, though not be destroyed, by admixture with such emanations from the body as sweat and carbon dioxide. It is well established that sweating conditions reduce the protection period with most, if not all, repellents, but this reduction has been attributed to increased attractiveness of the host, increased evaporation or absorption of the repellent, and dilution of the repellent.

Since in practical use much of the repellent is rubbed off the skin by contact with the clothing or other objects, abrasion probably constitutes the principal mode of loss. The loss by abrasion is subject to extreme variation, depending on the activity of the user. Abrasion is avoided in most experimental evaluations, where the arms are protected from rubbing in order to compare other aspects of the effectiveness of the repellents. However, complete evaluation of a repellent (Smith *1958*) should include studies of its resistance to loss by abrasion. Gilbert et al. (*1957*) have shown that repellents vary widely in this attribute. They reported that diethyltoluamide withstood 2 to 4 times as much wiping as ethyl hexanediol (ibid.) and at least 10 times as much as dimethyl phthalate (unpublished data).

MATERIALS AND METHODS

The repellents used in these studies were dimethyl phthalate, ethyl hexanediol, and deet (*N,N*-diethyl-*m*-toluamide, technical, about 95 percent meta isomer). They are representative of three chemical groups, and all have been extensively tested against a large number of species under many different conditions.

All tests were made with the yellow fever mosquito (*Aedes aegypti* (L.)) from a colony that has been maintained at the laboratory for many years. The species is easy to rear and populations in test cages maintain a uniform biting rate over a longer test period than the common malaria mosquito (*Anopheles quadrimaculatus* Say), another species colonized at the laboratory. Studies by Gouck and Smith (*1962*) showed that the avidity of caged populations of mosquitoes when exposed to marginal concentrations of repellents increased rapidly with age up to 6 days and was more uniform thereafter, but at all ages avidity was much lower each morning than during the previous afternoon. To obtain the greatest uniformity, 7- to 8-day-old mosquitoes were used in these studies, and dosages were adjusted so that tests could be completed in a half day whenever possible. Except as noted, all tests were run in the morning.

Most of the tests on human subjects were conducted with six Caucasian men, designated as subjects A through F. In some studies two young Caucasian women, designated G and H, also served as subjects.

Biting-rate tests were made to determine the relative attractiveness of different subjects to the mosquitoes when no repellent was on the skin. In preparing for these tests each subject put his arm into a stock cage infested with a large number of mosquitoes and allowed about 50 to start biting. He then removed his arm carefully and deposited the mosquitoes in a test cage. Each subject performed this task twice. This assured a test cage stocked with avid mosquitoes not too numerous for accurate counts. In conducting a test two subjects exposed their right arms simultaneously and then their left arms simultaneously in the test cage. Counts of biting mosquitoes were made at the end of 1 minute. Each test consisted of two exposures. The results were averaged in computing the counts on the basis of bites per square inch. When three subjects were participating, the tests were run in a round-robin series, or incomplete block design. Each subject paired arms with each other subject, as shown in figure 1, for an equal number of times.

FIGURE 1.—Biting-rate test, in which two subjects expose untreated arms in a cage of mosquitoes and count the mosquitoes that bite in 1 minute.

In protection tests the repellents were applied at full strength or at various dilutions in ethanol to measured areas of the forearms of subjects. The hands were protected by cotton gloves. The arms were exposed in cages of mosquitoes for 3 minutes, or until a confirmed bite (a bite followed by another in the same or the subsequent exposure period) or a larger given number of bites was received, as shown in figure 2. The required number of bites and the intervals between exposures varied from one experiment to another. The repellents were applied from individually calibrated pipettes and spread with a glass rod. The repellent remaining on the rod was recovered by rinsing with alcohol. The amount recovered was determined by spectrophotometric measurement and was subtracted from the amount delivered from the pipette to determine the actual amount applied, which varied slightly from the intended dosage. In most experiments the tests were conducted in one or more round-robin series.

To determine the rate of evaporation from various surfaces, measured areas of the forearms of human subjects, guinea pigs, or pieces of cloth were treated with the repellents at full strength or at various concentrations in ethanol and exposed in evaporation chambers, which consisted of two 8-liter conical percolators placed horizontally in a rack, as shown in figure 3. The large end of each percolator was closed by a plastic disk, perforated with a circle of twelve 3/32-inch

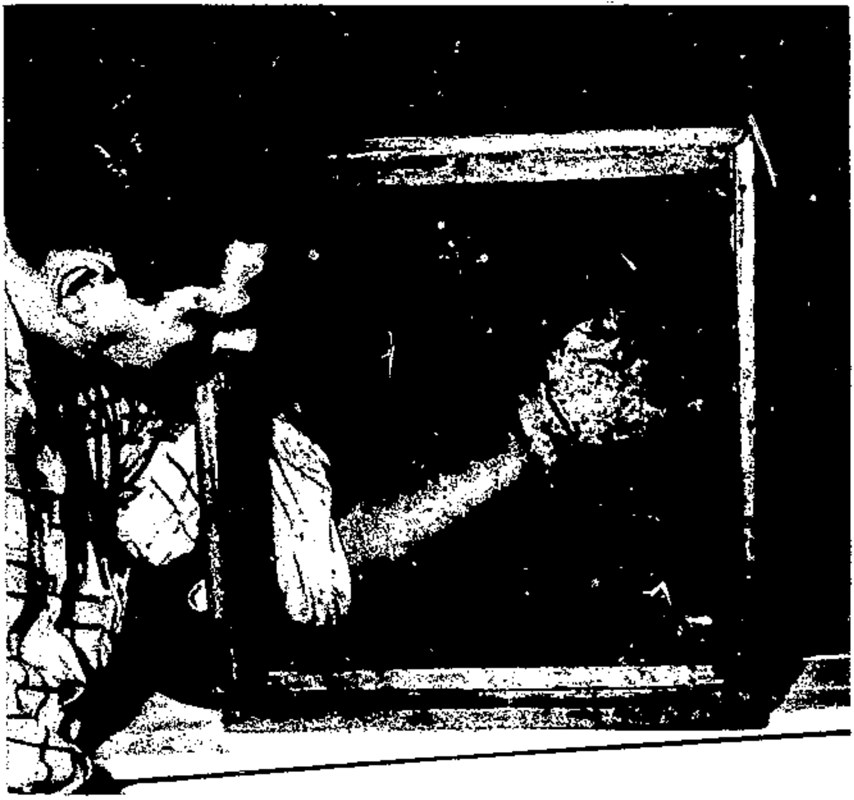

FIGURE 2.—Exposing an arm treated with repellent in a cage of *Aedes aegypti*. Some of the mosquitoes that appear to be on the arm may actually be on the wire screen between the arm and the camera.

holes to admit air. In tests with treated arms the disk had a central orifice with a Monel [3] metal sleeve to permit entry of the arm. In tests with cloth it had two small openings to permit the passage of water tubes. The small end of each percolator was connected by ⅜-inch copper tubing to a series of two 500-ml. and two 250-ml. gas-washing bottles, which in turn were connected to a vacuum pump, which maintained a flow of air through the system.

The air flow was adjusted to a rate of 26 liters per minute, as measured by a flowmeter inserted in the system directly following the percolator. During all test periods air was constantly passing over the enclosed arm, guinea pig, or cloth, out the small end of the vessel, and through ethanol in the gas-washing bottles, where the evaporating repellent was collected. A thermometer in the percolators was used to observe the temperature, which varied between 85° and 87° F.

In the skin tests a treated and an untreated arm of a subject were enclosed in the percolators immediately after the repellent had been applied, and the Monel metal sleeves were sealed to the upper arms with adhesive tape. Air was drawn over the treated arm for 2 hours,

[3] The mention of proprietary products does not constitute their endorsement by the U.S. Department of Agriculture.

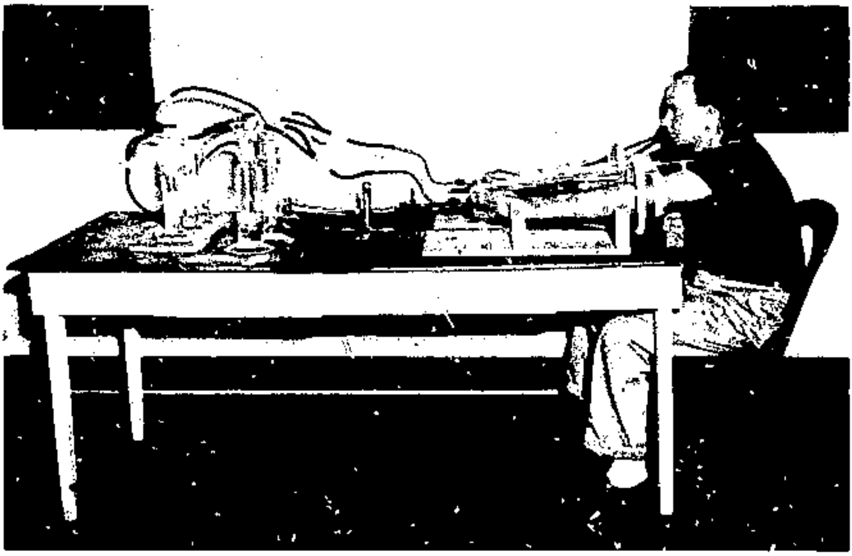

FIGURE 3.—Equipment for collecting the repellent lost by evaporation. Air is drawn by a vacuum pump over the treated arms of the subject and through ethanol in the gas-washing bottles, where the repellent is collected.

immediately after which the repellent remaining on the arm was recovered by rinsing it with 500 ml. of distilled ethanol, as shown in figure 4. The same procedure was used on untreated arms to establish blanks for the spectrophotometric readings. After a large number of tests had demonstrated that the blank readings were negligible, treated arms of two subjects were exposed simultaneously, and tests were conducted in round-robin series.

The percolators were rinsed with ethanol to remove any adhering repellent. The amounts of repellent in the ethanol from the gas-washing bottles and rinsed from the arm and percolator were determined spectrophotometrically. The methods of Schmidt et al. (*1958*) for deet and of Bowman et al. (*1959*) for ethyl hexanediol were used. Dimethyl phthalate was read at 225 mμ. All measurements were computed in terms of milligrams of repellent per square inch of skin.

The guinea pigs were restrained in a rack in a supine position, and a 7.5-square-inch area on the shaved ventral surface was treated with the repellent. Other test procedures were the same as those with treated arms, except that 4-hour evaporation periods were used in some tests.

Cloth tests were made with sleeves of cotton sheeting 9 inches in circumference and 7½ inches long. The sleeves were washed and extracted to remove ethanol-soluble impurities and then stretched over glass cylinders 2¾ inches in diameter and 11 inches long. The repellent was applied in a 5-percent ethanol solution to assure thorough saturation of the cloth. Cylinders with treated and untreated cloth were placed in the percolators and maintained at a temperature of 95° F. with warm water, which was circulated through the cylinders by means of tubes passing through the disks closing the percolators.

FIGURE 4.—Rinsing a treated arm with ethanol to remove the repellent remaining after a test for measurement of the residue by ultraviolet spectrophotometry. The man at the left is blowing a fine stream of ethanol from a washing bottle over the treated arm, from which it runs through the funnel into the flask.

After a 2-hour evaporation period, the sheeting was cut from the cylinder and the remaining repellent was recovered by extraction with ethanol in a Soxhlet apparatus.

The amount of repellent absorbed into the skin of the arm or the guinea pig was determined by subtracting the amount lost by evaporation and the amounts rinsed from the skin and percolators from the total amount applied. Less than 5-percent error was incurred in this method, as demonstrated in studies with guinea pigs by Schmidt et al. (*1959*) with C^{14}-labeled deet. Radioactivity equivalent to 95–96 percent of the dosage calculated to be absorbed was recovered in the urine, feces, and samples of the skin and hair, although some activity still remained in the last two, and the total recovery reached 98–99 percent of the applied dosage.

A balanced, incomplete block design, or round-robin series, was employed in most experiments, except those with a range of dosages. With this design each repellent, subject, or experimental condition in the series was paired against each other repellent, subject, or condition. An adjusted average, which compensates for variation between hosts and testing conditions, was computed by a statistical method suggested by J. U. McGuire of the Entomology Research Division and modified from Kempthorne (*1952*). From the analysis of variance the least significant difference at the 5-percent level between any two repellents, subjects, or conditions was determined. In some experiments the various repellents, subjects, or conditions were tested in a series of pairs, rather than in a single integrated design. In such experiments the significant difference between means was computed from the "t" value of the standard error of the mean difference.

FATE OF REPELLENTS ON SKIN AND CLOTHING

Repellents are effective for much longer periods when applied to the clothing than when applied to the skin and persist for days or weeks rather than hours. At the beginning of these studies it was assumed that losses by evaporation from skin and clothing would be comparable, but that skin applications would also be subject to losses by absorption and deterioration. Experiments were made with applications to the arms, guinea pigs, and cloth to determine loss by evaporation, to the arms and guinea pigs to determine loss by absorption, and to the arms only to determine loss by deterioration.

Physical Loss

A preliminary series of tests was conducted to determine the amounts of dimethyl phthalate lost by evaporation in 2 hours from heavy and medium applications on the arms of six subjects. The averages of the results obtained were as follows:

Amount—	*Heavy*	*Medium*
Applied (mg. per sq. in.)	13.00 ± 0.71	7.40 ± 0.42
Evaporated (mg. per sq. in.)	.60 ± .06	.61 ± .06
Evaporated (percent)	4.6	8.2

The loss by evaporation was almost identical at both dosages when expressed in milligrams per square inch of skin surface and twice as great at the medium rate as at the heavy rate when expressed as a percentage of the amount applied. Kasman et al. (*1953*) likewise noted that the evaporation of dimethyl phthalate from filter paper depended on the surface area and not on the amount of repellent applied. From these results it is apparent that percentage losses are relative, whereas losses per unit of surface are definite, and the latter value was used throughout the remainder of these studies.

Additional tests were made to determine the individual variation in evaporation rates on six subjects treated with dimethyl phthalate at a uniform rate. The loss in 2 hours was fairly uniform, ranging from 0.52 to 0.64 mg. per square inch, as shown in table 1.

A series of tests was conducted to determine the total amounts of three repellents lost from the arms over various periods of time when the subjects were free to move about in a room maintained at about 80° F. The repellents were applied at accurately measured dosages between 6.75 and 7.25 mg. per square inch. After periods of 5, 30, 60, 120, and 240 minutes the arms were rinsed with distilled alcohol to remove the repellent, which was determined quantitatively by spectrophotometry. The loss after 5 minutes was taken as representing the amount of repellent unrecoverable immediately after application. This amount was subtracted from the total lost in each of the other periods in calculating the amounts lost per square inch per minute. Each test was replicated six or eight times and twice on each of three or four subjects. The same subjects were not used with every repellent. The average results of this first series are shown in table 2.

A slight decline in the rate of loss per minute with increasing length of the exposure period was evident in this series. Over the longer periods dimethyl phthalate showed the highest rate of loss and ethyl hexanediol the lowest, although the differences were small.

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TABLE 1.—*Evaporation of dimethyl phthalate from arms of 6 subjects during 2 hours in glass percolators. (Air flow 26–27 liters per minute; average of 2 tests)*

Subject	Surface of arm treated	Amount of repellent per square inch	
		Applied	Evaporated
	Sq. in.	*Mg.*	*Mg.*
A	62.9	7.77	0.64
B	70.6	6.89	.55
C	75.3	6.93	.61
D	69.3	6.99	.57
E	75.7	6.65	.52
F	66.1	6.97	.57
Average	69.98	7.032±.131	.575±.016

A similar series was conducted in which each repellent was applied to the same three subjects. The results of this second series are shown in table 2.

There was relatively little difference between the rates of loss on the different subjects and no consistent difference. In this series of tests the losses were, in general, slightly lower than in the preceding series. The loss of deet after 30 minutes was unexplainably low for all three subjects, and there was less trend toward decreased rates of loss with longer exposure periods.

In a third series of tests the repellents were applied to two female subjects. The average individual losses in two tests are shown in table 2.

There were no consistent differences in the rates of loss between the two subjects. In general, losses were slightly higher than in the two preceding series on men, but were not consistently so. The overall average losses in milligrams per square inch per minute were 0.010 for dimethyl phthalate, 0.009 for deet, and 0.008 for ethyl hexanediol.

The losses by evaporation and absorption of repellents applied to three male and two female subjects, guinea pigs, and cloth were investigated in eight series of tests. The results are presented in table 3.

The first two series were conducted to compare the losses of dimethyl phthalate and deet on subjects A and C, who had shown long and short protection periods, respectively (see p. 18). The results for both subjects were very similar. Dimethyl phthalate evaporated about twice as fast as deet, but the latter was more rapidly absorbed, and the total losses of both repellents were about the same. In the third series the losses of ethyl hexanediol on these subjects and subject B were determined. Again the losses by evaporation for the three subjects were similar, but there were slightly larger differences in absorption. The rate of evaporation was similar to that of dimethyl phthalate, but absorption and total loss were generally lower, except for subject C.

In series 4 the losses of all three repellents were compared on two women, subjects G and H, and in series 5 on the two women and a

TABLE 2.—*Evaporation per minute of 3 repellents from arms of 5 subjects during various intervals after treatment in 3 test series. (6.75–7.25 mg. per sq. in. applied)*

SERIES 1 (6–8 TESTS)

Repellent and subject [1]	Amount of repellent evaporated per square inch at indicated interval after treatment			
	30 minutes	60 minutes	120 minutes	240 minutes
	Mg.	*Mg.*	*Mg.*	*Mg.*
Dimethyl phthalate	0.010	0.011	0.009	0.009
Deet	.014	.010	.008	.007
Ethyl hexanediol	.010	.010	.005	.006

SERIES 2 (1 TEST)

Repellent and subject [1]	30 minutes	60 minutes	120 minutes	240 minutes
Dimethyl phthalate:				
A	0.015	0.005	0.006	0.008
B	.011	.006	.007	.007
C	.016	.005	.006	.006
Deet:				
A	.001	.007	.006	.005
B	.001	.007	.004	.006
C	.001	.005	.004	.005
Ethyl hexanediol:				
A	.010	.011	.004	.006
B	.008	.009	.005	.006
C	.013	.009	.005	.005

SERIES 3 (2 TESTS)

Repellent and subject [1]	30 minutes	60 minutes	120 minutes	240 minutes
Dimethyl phthalate:				
G	0.012	0.009	0.009	0.011
H	.016	.008	.011	.007
Deet:				
G	.015	.009	.010	.011
H	.018	.011	.008	.011
Ethyl hexanediol:				
G	.009	.008	.008	.011
H	.005	.008	.008	.008

[1] In series 1 the results with three or four subjects were pooled.

man, subject B. Rates of evaporation in milligrams per square inch were similar on all subjects for deet (0.25–0.29), slightly less for dimethyl phthalate (0.48–0.57), and still more diverse for ethyl hexanediol (0.33–0.46). Losses by absorption generally showed greater variation between subjects—0.32–0.52 for dimethyl phthalate, 0.43–0.88 for deet, and 0.30 to 0.43 for ethyl hexanediol. Total losses per minute were about the same for dimethyl phthalate and deet, averaging 0.008, and were slightly lower for ethyl hexanediol (0.006).

Losses of dimethyl phthalate and deet from guinea pigs were determined in series 6. There was more variation between individual guinea pigs than between individual human subjects, and losses were higher on guinea pigs by both evaporation and absorption. Dimethyl

TABLE 3.—*Evaporation, absorption, and loss per minute of 3 repellents applied to human subjects, guinea pigs, and bleached muslin during 2 hours in glass percolators in 8 test series. (Applied as 30-percent solutions in ethanol)*

SERIES 1 (6 TESTS)

Repellent and subject	Amount of repellent per square inch			
	Applied	Evaporated	Absorbed	Lost per minute
Dimethyl phthalate:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
A	7. 02	0. 54	0. 48	0. 009
C	7. 01	. 53	. 54	. 009
SERIES 2 (5 TESTS)				
Deet:				
A	7. 03	0. 24	0. 80	0. 009
C	6. 98	. 24	. 77	. 008
SERIES 3 (3 TESTS)				
Ethyl hexanediol:				
A	7. 03	0. 57	0. 30	0. 007
B	7. 46	. 52	. 45	. 008
C	7. 01	. 55	. 52	. 009
SERIES 4 (2 TESTS)				
Dimethyl phthalate:				
G	6. 60	0. 54	0. 52	0. 009
H	7. 16	. 50	. 40	. 008
Deet:				
G	6. 53	. 25	. 43	. 006
H	6. 89	. 29	. 49	. 007
Ethyl hexanediol:				
G	6. 43	. 36	. 30	. 006
H	6. 63	. 33	. 34	. 006
SERIES 5 (4 TESTS)				
Dimethyl phthalate:				
G	6. 51	0. 57	0. 37	0. 008
H	6. 53	. 48	. 32	. 007
B	6. 52	. 57	. 39	. 008
Deet:				
G	6. 57	. 27	. 88	. 010
H	6. 47	. 27	. 84	. 009
B	6. 82	. 29	. 67	. 008
Ethyl hexanediol:				
G	6. 12	. 33	. 42	. 006
H	6. 09	. 38	. 43	. 007
B	5. 99	. 46	. 37	. 007

TABLE 3.—*Evaporation, absorption, and loss per minute of 3 repellents applied to human subjects, guinea pigs, and bleached muslin during 2 hours in glass percolators in 8 test series. (Applied as 30-percent solutions in ethanol)*—Continued

Repellent and subject	Amount of repellent per square inch			
	Applied	Evaporated	Absorbed	Lost per minute
SERIES 6 (1-2 TESTS)				
Dimethyl phthalate, guinea pig:	*Mg.*	*Mg.*	*Mg.*	*Mg.*
A1	7.80	0.87	1.71	0.022
A2	8.00	.62	.56	.010
A3	8.00	.78	2.40	.026
A4	7.89	1.01	2.14	.026
Deet, guinea pig:				
B1	6.72	.35	1.00	.011
B2	6.12	.39	1.18	.013
B3	6.48	.33	.90	.010
B4	6.47	.35	1.10	.012
C1	7.85	.94	1.95	.024
C2	8.00	.62	.56	.010
C3	8.00	.78	2.40	.027
SERIES 7 (1 TEST) [1]				
Deet, guinea pig:				
C1	6.38	0.66	1.68	0.010
C2	6.31	.74	1.70	.010
C3	6.28	.65	1.51	.009
C4	6.14	.66	1.48	.009
SERIES 8 (6 TESTS)				
Dimethyl phthalate, muslin	6.96	0.23		
Deet, muslin	6.94	.11		
WEIGHTED AVERAGES				
Dimethyl phthalate:				
Human subjects		0.54	0.44	0.008
Guinea pigs		.82	1.70	.021
Muslin		.23		
Deet:				
Human subjects		.26	.74	.008
Guinea pigs		.36	1.05	.012
Muslin		.11		
Ethyl hexanediol, human subjects		.44	.40	.007

[1] 4-hour exposure.

phthalate evaporated about twice as fast as deet. In series 7 guinea pigs treated with deet were retained in the glass percolators for 4 hours instead of 2. The average losses by evaporation were about double those for 2 hours (0.678 vs. 0.355), showing that the rate remained constant throughout this period even though the amount on the skin had decreased by about one-third. Losses by absorption were only about 50 percent larger than at 2 hours (1.59 vs. 1.05).

Losses by evaporation from cloth were determined in series 8. Dimethyl phthalate was lost twice as fast as deet, but both were lost only half as fast as by evaporation from human subjects and one-third as fast as from guinea pigs.

The weighted-average losses are also given in table 3 for all the 2-hour tests on human subjects, guinea pigs, and cloth.

A test was made to determine the evaporation rate of deet from a guinea pig over a 24-hour period. The ethanol traps in which the evaporated repellent was collected were changed every 3 hours. A total of 46.66 mg., or 6.22 mg. per square inch, was applied. The amounts lost by evaporation during each 3-hour interval after treatment were as follows:

Hours	*Total amount lost*	*Amount lost per square inch*
	Mg.	*Mg.*
1–3	2.34	0.312
3–6	2.35	.312
6–9	2.60	.345
9–12	2.43	.324
12–15	2.88	.384
15–18	.80	.108
18–21	.47	.063
21–24	.34	.045

The evaporation rate was essentially constant over the first 12 hours, increased slightly during the next 3 hours, then decreased sharply, and continued to decrease more slowly. Since the rate of absorption has been shown to exceed that of evaporation by two or three times during the first 4 hours (table 3, series 6 and 7), it seems probable that very little repellent was left on the skin after 15 hours, when the evaporation rate first declined.

The preceding tests, and others mentioned previously, indicate that the rate of evaporation of a repellent from the skin remained fairly constant under uniform environmental conditions even though the dosage or amount remaining per square inch might be changed, as long as the dosage remained high. Tests were made to compare the rates of evaporation of three repellents at high and low dosages on each of two human subjects.

A 6-mg. dosage per square inch was paired with a 1-mg. dosage on opposite arms of the same subject, and a 12-mg. dosage was paired with 0.5 mg. To obtain the different dosages, different concentrations of repellent in ethanol were used, since it was impossible to cover the arms adequately with the full-strength repellent at the lower dosages. The two arms of the subject were exposed simultaneously in the glass percolators. The gas-washing bottles used to collect the dimethyl phthalate and deet were changed every half hour for 2 hours. Those used to collect the ethyl hexanediol were only removed at the end of the 2-hour test period. The repellent remaining on the arms at the end of the 2-hour test period was recovered by rinsing and the amount absorbed during the entire 2 hours was computed.

The results are given in table 4. At all dosages deet was lost about half as fast as the other repellents. There was some increase in the amount of each repellent lost with each increase in the dosage applied, but the amount lost did not represent a constant percentage of the amount applied. For example, 60 percent of the 0.5-mg. dosage of dimethyl phthalate was lost during 2 hours as opposed to only 5 percent of the 12-mg. dosage. The rate of loss usually remained fairly constant for each half-hour period, but at the 0.5-mg. dosage it declined in each successive period.

TABLE 4.—*Evaporation and absorption of 3 repellents during various intervals after treatment from arms of 2 subjects treated with various dosages. (Average of 2 tests on each subject; all amounts in mg. per sq. in.)*

Repellent and dosage	Amount of repellent evaporated in indicated interval after treatment				Total evaporated for subject—		Total absorbed for subject—	
	1–30 minutes	31–60 minutes	61–90 minutes	91–120 minutes	A	C	A	C
Dimethyl phthalate:								
12	0.169	0.170	0.163	0.160	0.62	0.70	0.78	0.95
6	.141	.141	.134	.135	.53	.57	.82	.84
1	.102	.091	.084	.086	.32	.40	.55	.66
.5	.096	.085	.064	.062	.30	.31	.39	.40
Deet:								
12	.078	.080	.075	.076	.29	.32	1.13	1.39
6	.075	.073	.071	.075	.26	.32	.56	.80
1	.048	.047	.044	.047	.20	.17	.41	.35
.5	.049	.039	.034	.032	.16	.15	.28	.26
Ethyl hexanediol:								
12					.62	.63	.73	.86
6					.63	.61	.66	.86
1					.40	.46	.40	.52
.5					.23	.27	.31	.30

Deterioration on Skin

Studies were conducted to determine whether the repellents deteriorated during a period of aging on the skin, either by absorption of skin secretions that reduced their effectiveness or by chemical breakdown.

Since the skin gives off carbon dioxide, and carbon dioxide attracts mosquitoes at certain concentrations, tests were made to determine whether a decrease in the effectiveness of a repellent was caused by passing carbon dioxide through it. Carbon dioxide was passed through 100 ml. of dimethyl phthalate for 1, 2, 4, and 16 hours at the rate of 2 liters per minute. Protection tests with the repellent immediately after removal of the carbon dioxide flow showed no decrease in effectiveness in comparison with untreated dimethyl phthalate. Carbon dioxide determinations were made immediately after removing the repellent from the flow and after 20 and 45 hours at room temperature. The repellent became saturated in 16 hours or less and lost carbon dioxide rapidly after standing in open containers at room temperature.

A test with carbon dioxide passing through water-free and water-saturated dimethyl phthalate for 1 hour showed no biological differences on cotton stockings, skin, or artificial membranes, or in blood supplying the artificial membranes. The method for testing on cotton stockings is given by Smith (*1958*) and that on membranes or in blood by Bar-Zeev and Smith (*1959*). The failure to obtain biological differences may be caused by the rapid evaporation of the carbon dioxide when applied to cloth, skin, artificial membrane, or blood.

Tests were conducted to determine the effect of adding sweat to three repellents. Sweat was collected from the arms of each of three subjects. Ethanol solutions containing equal parts of sweat and repellent were tested on one arm of the same subject from which it was collected and paired with an equal concentration of the repellent alone on the other arm. The average protection times to the first confirmed bite are given in table 5. The addition of sweat caused no consistent reduction in the effectiveness of the repellent, although there was a significant reduction with deet on subject C.

TABLE 5.—*Protection time with ethanol solutions of 3 repellents, alone and mixed with equal concentrations of sweat, on 3 human subjects against* Aedes aegypti. (*4 tests*)

Repellent (concentration and amount per forearm) and subject	Protection time		
	Without sweat	With sweat	Ratio
	Minutes	*Minutes*	
Dimethyl phthalate (25 percent, 2-4 ml.):			
A	100	89	0.89
B	140	136	.97
C	21	14	.67
Deet (10 percent, 1 ml.):			
A	353	385	1.09
B	373	371	.99
C	339	246	[1] .73
Ethyl hexanediol (25 percent, 1 ml.):			
A	294	263	.89
B	254	245	.96
C	211	238	1.13

[1] Difference significant at 5-percent level.

To determine whether bacteria on the skin contribute to decreasing the effective period, dimethyl phthalate was tested concurrently on unwashed arms and on arms that had been washed twice with a 1:1,000 solution of merthiolate and rinsed twice with alcohol. The merthiolate solution alone did not prevent biting. The period of protection was almost identical on the aseptic and nonaseptic arms of four sub-

TABLE 6.—*Protection time with aseptic and nonaseptic arms of 5 subjects treated with 1 gm. of dimethyl phthalate against* Aedes aegypti

Subject	Protection time on—			
	Aseptic arm to—		Nonaseptic arm to—	
	2 bites	5 bites	2 bites	5 bites
	Minutes	*Minutes*	*Minutes*	*Minutes*
A	225	225	225	253
C	187	247	68	248
D	128	158	128	158
E	155	155	155	155
F	270	270	272	272

jects and slightly longer on the nonaseptic arm of a fifth subject. The detailed results are given in table 6.

An additional series of tests was conducted to determine whether a repellent treatment failed to give protection after a period of aging on the skin solely because of the quantity lost or whether the remaining repellent had deteriorated and was therefore less effective than an equal amount of repellent freshly applied. Dosages of deet small enough to permit completion of a test in a single morning were applied to the right forearms of each of two subjects. The subjects alternately exposed their treated arms in the same cage of mosquitoes until five bites were received in 3 minutes. The forearms were immediately rinsed with 500 ml. of distilled alcohol and a quantitative determination was immediately made by ultraviolet spectrophotometry. Fresh applications of deet in the amount recovered from the arms were then made to the left forearms of the same subjects, which were immediately tested in the same cage of mosquitoes.

The results are given in table 7. The amount of repellent recovered immediately after five bites were received ranged from 0.33 to 0.50 mg. per square inch for subject A and from 0.36 to 0.44 mg. for subject C. When approximately equal amounts were applied to the other arm, A received five bites immediately in two tests and five bites after 30 minutes in two others; C received five bites immediately in three tests and five bites after 60 minutes in another. The MED in this series of tests was therefore between 0.30 and 0.50 mg. per square inch, and freshly applied repellent was no more effective than an equal amount of repellent remaining as an aged residue from a heavier original application.

In a further effort to determine whether any chemical breakdown occurred during the time the repellent was aging on the skin, samples of recovered repellent were analyzed by infrared spectrophotometry. One arm of subject A was treated with deet and one arm of subject C with dimethyl phthalate. After 6 hours of aging the repellents were recovered by rinsing the arms with ethanol, and the untreated arms were also rinsed with ethanol to provide a blank or background reading. Through the kindness of S. A. Hall and Morton Beroza of the Entomology Research Division, Beltsville, Md., these solutions were

TABLE 7.—*Effectiveness of fresh applications of deet to left arms of 2 subjects in amounts equal to those recovered by rinsing from right arms immediately after receiving 5 bites from caged* Aedes aegypti. *(All amounts in mg. per sq. in.)*

Subject and amount applied to right arm	Time to 5 bites	Amount recovered from right arm	Amount applied to left arm	Time to 5 bites
A:	*Minutes*			*Minutes*
0.93	121	0.50	0.50	Immediately.
0.80	121	.38	.43	30.
0.80	180	.33	.34	30.
0.64	30	.41	.43	Immediately.
C:				
0.80	122	.36	.37	Do.
0.79	90	.44	.46	Do.
0.77	122	.39	.40	60.
0.53	30	.36	.39	Immediately.

compared with standard solutions of the fresh repellents. The infrared curves indicated that there had been no appreciable change in either repellent.

Excretion in Urine

The possibility of recovering unchanged repellent from the urine of treated subjects was investigated. Known amounts of repellent were added to urine samples. A 100-ml. sample of urine was shaken up with equal parts of isooctane for 5 minutes to mix thoroughly. A 50-ml. sample of the mixture of isooctane and urine was centrifuged to separate the isooctane from the solids. The clear sample of isooctane was run on the ultraviolet spectrophotometer. The amount of deet recovered from the first extraction was about 74 percent and that of dimethyl phthalate about 100 percent of the amount used. Samples of urine from the untreated subjects were collected over a 24-hour period and pooled. A check sample was run on the spectrophotometer from these collections.

The forearms were then treated with about 18 mg. of deet or dimethyl phthalate, which remained on the arms for 6 hours. Urine samples collected for 24 hours after treatment and also samples for the 48-hour period after treatment were analyzed for the repellents by spectrophotometry. No differences were observed between the readings of the treated and check samples on the spectrophotometer, indicating that no deet or dimethyl phthalate in its original form was present in the urine, or that the amount eliminated unchanged was too small for determination.

These results were confirmed in subsequent studies by Schmidt et al. (*1959*) with C^{14}-labeled deet applied to guinea pigs. Their findings demonstrated that, although most of the absorbed radioactivity was excreted in the urine, no unchanged deet was present.

Kasman et al. (*1953*) found evidence that a metabolite of the repellent 1-phenyl-2-hydroxypropanone-1 was present in considerable quantity in the urine from a treated guinea pig. They concluded that absorption was a significant factor in repellent loss.

FACTORS AFFECTING PROTECTION PERIOD

The length of time any given dosage of a repellent remains effective depends not only on the rate at which it is lost from the skin but also on the minimum residual amount that will continue to prevent biting, a factor that has sometimes been overlooked. For example, Kasman et al. (*1953*) found that dimethyl phthalate was lost by evaporation from filter paper at 0.00044 mg. per square centimeter per minute. From this, assuming that it would evaporate at the same rate from a guinea pig, they calculated that the protection time should be 3,900 minutes from a dosage of 1.7 gm. per square centimeter if evaporation were the only mode of loss, a computation that also assumes that the repellent would give protection as long as any appreciable quantity remained on the skin.

The studies conducted at Orlando, however, showed that this is a minimum effective dosage, or MED, which will presumably be affected by such factors as the avidity of the insects and the attractiveness of the host, as well as the efficiency of the repellent. Variations in the avidity of the mosquitoes as a factor in the MED were eliminated as much as possible by the procedures described under Materials and Methods. Studies on the effect of the host on the protection obtained included experiments on the amounts of repellent remaining on different subjects at the time the first bites were received, the amounts required to provide protection when first applied, correlations between attractiveness of the host without repellent and the protection received, the effect of hair and sebum on attractiveness and protection, and the relative effectiveness of repellents on humans and animals.

Residual Effectiveness and Rate of Loss in Relation to Protection Period

Two series of tests were conducted to determine the amounts of dimethyl phthalate that remained on the arms of six subjects at the time the repellency dropped to a level that allowed five bites in a 3-minute exposure. In the first series paired tests were made in an incomplete block design that consisted of three tests with each arm of each subject. This experiment was made early in the general study before the technique of recovery of repellent from the arms by rinsing had been developed. As soon as five bites were received, recovery was made by wiping the arms with ethanol-soaked cotton pads and dry pads and extracting the repellent from the pads. This technique proved to be less efficient than rinsing, and the actual amounts of repellents remaining on the arms were probably slightly higher than the amounts measured. However, the data are presented in table 8, as they provide a valid comparison of the differences and similarities between subjects.

The average protection times ranged from 52 to 200 minutes on different subjects, with an overall average of 148 minutes. The recovery rates ranged from 4.10 to 5.68 mg. per square inch, with an average of 4.49. Loss per square inch per minute ranged from 0.010 to 0.020 mg., with an average of 0.015. Five subjects had almost identical amounts of repellent remaining on the arms when five bites were received (4.10 to 4.43 mg.), although their protection times

TABLE 8.—*Protection time with dimethyl phthalate on 6 human subjects against* Aedes aegypti, *amount of repellent recovered by wiping after 5 bites were received, and calculated rate of loss. (Average of 7 mg. per sq. in. applied; 6 tests)*

Subject	Protection time	Amount of repellent per square inch		
		Recovered	Lost	Lost per minute [1]
	Minutes	*Mg.*	*Mg.*	*Mg.*
A	200	4.43	2.57	0.010
B	172	4.23	2.81	.013
C	99	4.37	2.50	.020
D	157	4.10	2.91	.015
E	52	5.68	1.34	.015
F	147	4.15	2.84	.016
Average	148	4.49	2.49	.015

[1] 0.54 mg. per square inch was unrecoverable by this method immediately after application, and this amount was subtracted from the total lost before the loss per minute was computed.

showed a twofold range (99 to 200 minutes). The protection time was inversely correlated with the rate of loss. Subject E received bites with a higher residual dosage of repellent than the others and had the shortest protection period, even though he lost repellent at the average rate.

A second more extensive series of tests was made with subjects A and C, who had shown long and short protection times, respectively, to confirm the differences observed in the first series and to determine whether there was a significant difference between the protection times on opposite arms of the same subject.

Six paired tests were run. First the right arm of each subject, then the left arm of each, was exposed in a single cage at each exposure period. New cages were used for every exposure period to assure fresh mosquitoes. The repellent was wiped from the arms immediately after it became ineffective, that is, when five bites were allowed in 3 minutes. The results are given in table 9.

Again there were significant differences in the protection time and loss per minute between subjects, but not between opposite arms of the same subjects. Subject A, with the longest protection period, lost 0.011 mg. per square inch per minute from the right arm and 0.013 from the left, about the same as in the previous test series (0.010). Subject C, with the shortest protection period, lost 0.028 and 0.023 mg. per square inch per minute, slightly more than in the previous test (0.020). However, in this series there was also a statistically significant, though small, difference in the average amount recovered from the arms, as subject A received bites with more repellent on his arms than subject C.

TABLE 9.—*Protection time with dimethyl phthalate on opposite arms of 2 subjects against* Aedes aegypti, *amount of repellent recovered, and rate of loss. (Average of 7 mg. per sq. in. applied)*

Subject and test No.	Right arm: Protection time	Right arm: Amount of repellent per square inch: Recovered	Right arm: Amount of repellent per square inch: Lost	Right arm: Amount of repellent per square inch: Lost per minute	Left arm: Protection time	Left arm: Amount of repellent per square inch: Recovered	Left arm: Amount of repellent per square inch: Lost	Left arm: Amount of repellent per square inch: Lost per minute [1]
A:	*Minutes*	*Mg.*	*Mg.*	*Mg.*	*Minutes*	*Mg.*	*Mg.*	*Mg.*
1	120	5.37	1.68	0.010	120	4.87	2.13	0.013
2	150	4.70	2.35	.012	180	4.49	2.51	.011
3	270	4.29	2.73	.008	300	4.07	2.95	.008
4	120	4.85	2.16	.014	120	4.59	2.37	.015
5	120	4.85	2.13	.013	120	4.74	2.31	.015
6	120	5.14	1.88	.011	120	4.89	2.13	.013
Average	150	4.87	2.16	.011	160	4.61	2.40	.013
C:								
1	60	4.68	2.35	.030	120	4.49	2.54	.017
2	120	3.51	3.54	.025	120	3.81	3.24	.023
3	210	3.35	3.68	.015	180	4.02	2.96	.013
4	30	5.02	2.00	.049	30	4.96	2.02	.049
5	90	4.14	2.85	.026	120	4.48	2.52	.017
6	90	4.62	2.37	.020	90	4.75	2.27	.019
Average	100	4.22	2.80	.028	110	4.42	2.59	.023

[1] 0.54 mg. per square inch was unrecoverable by this method immediately after application, and this amount was subtracted from the total lost before the loss per minute was computed.

Attractiveness in Relation to Protection Period

Seven series of tests were made to study the attractiveness of five subjects to mosquitoes and the relationship of attractiveness to the protection period obtained with repellents. Attractiveness was measured by the biting-rate tests previously described (p. 2). The first series was conducted to determine the relative attractiveness of three subjects, A, B, and C, who had shown long, intermediate, and short protection periods, respectively, with dimethyl phthalate (table 8). Tests were made in the morning and afternoon with each arm of each subject in a round-robin series. The results are shown in table 10.

TABLE 10.—*Natural attractiveness of arms of 3 subjects to caged* Aedes aegypti. (*Average of 20 tests*)

Subject	Bites per square inch per minute				
	Left arm		Right arm		Average [1]
	A.M.	P.M.	A.M.	P.M.	
	Number	*Number*	*Number*	*Number*	*Number*
A	0.33	0.27	0.42	0.35	0.34
B	.79	.88	.88	1.07	.91
C	.31	.26	.32	.18	.26
L.S.D. at—					
1-percent level	.21	.19	.22	.19	.10
5-percent level	.15	.14	.17	.14	.08

[1] 80 tests.

Subject C, who had the shortest protection period, had fewer bites than subject A, who had the longest. However, the difference was not significant in the morning tests with either arm or with the left arm in the afternoon, and the difference in the overall average was just significant at the 5-percent level. Subject B was about three times as attractive as either A or C, but had protection periods intermediate between them. There was no consistent difference between the morning and afternoon biting rates. The overall average was 0.51 bite per square inch per minute in the morning and 0.50 in the afternoon. Gouck and Smith (*1962*) found greater avidity in mosquitoes tested against low concentrations of repellent in the afternoon than in the morning. However, in the tests reported here no repellent was used, and all the mosquitoes in the cages were selected as biters a few minutes before the tests were made.

Although these tests showed no correlation between the natural attractiveness of the subjects and the relative protection they had obtained with dimethyl phthalate, there was considerable variation in their biting rates from day to day and it seemed advisable to compare the two phenomena concurrently. Six round-robin series of tests were run, three with subjects A, B, and C, using three repellents, and three with subject A and two female subjects, G and H. The biting-rate tests and protection-time tests were made during the forenoons of the same days to assure identical avidity in both types of tests. As soon as the biting-rate tests were completed, the same measured area of the forearm was treated with repellent. The treated arm was then exposed to mosquitoes 5 minutes after treatment and every 30 minutes thereafter until a confirmed bite was received. The remaining repellent was rinsed from the arm with ethanol and determined spectrophotometrically, and the amount lost per square inch of skin per minute was calculated. The results are given in table 11.

In all tests comparing subjects A, B, and C (series 1a, 1b, and 1c), subject B showed the greatest natural attractiveness (highest biting rate) and subject C the least. The differences between B and A were always significant; those between A and C were significant in two of the three series. Subject C always had significantly shorter protection periods than B and A. He also lost repellent faster, significantly so in two of the three series. A received significantly longer protection than B with dimethyl phthalate only and showed no consistent difference in rate of loss. In these tests the failure of the repellents to protect C as long as the other subjects cannot be attributed to greater natural attractiveness, but it was apparently correlated with a faster rate of loss.

In the three series with subjects G, H, and A (2a, 2b, 2c), there was no correlation between the relative attractiveness of the subjects and the amount of protection received, and only incomplete correlation between the rate of loss and the protection time. Subject A, the man, was the least attractive in all three series of tests. The differences were statistically significant in two series, but they closely approached significance in the other series. In the series of tests with deet, A was significantly less attractive than G but not H, lost repellent faster than H but not G, and had a significantly shorter protection period than either G or H. With dimethyl phthalate, A did not lose repellent as fast as either G or H, but had a significantly shorter protection time than H. With ethyl hexanediol, A had the smallest rate of loss and the longest protection time. Subject H was less attractive than G in two series and more attractive in one, but always lost repellent more slowly and had longer protection periods. Subjects G and H were not compared directly with B and C, but comparisons may be made on the basis of their ratios to A, as given in table 11.

Additional experiments by Gouck and Bowman (*1959*) demonstrated that the arms of B, the most attractive subject, gave off the most carbon dioxide and the least water, those of C, the least attractive subject, the least carbon dioxide and the most water, and those of A, intermediate quantities. The application of repellents to the arms did not affect the release of moisture, but dimethyl phthalate, ethyl hexanediol, and deet reduced the carbon dioxide output of A and B, and deet reduced it on C also. However, no conclusive evidence indicated that the reduction of carbon-dioxide output resulted in the repellent action.

TABLE 11.—*Biting rate of* Aedes aegypti *and protection time with 3 repellents, including amount applied, recovered, and lost, on 5 human subjects in 6 test series. (4 tests)*

DIMETHYL PHTHALATE

Series and subject	Bites per square inch per minute	Amount of repellent per square inch			Protection time	
		Applied	Recovered	Lost per minute	Minutes	Ratio to subject A
	Number	*Mg.*	*Mg.*	*Mg.*		
Series 1a:						
A	0.53	7.42	5.98	0.025	76	1.00
B	1.97	7.30	6.15	.032	48	.63
C	.28	7.36	6.23	.038	18	.24
L.S.D. at 5-percent level	.22		.46	.021	25	
Series 2a:						
G	2.68	6.72	4.89	.020	82	1.22
H	3.23	6.71	4.52	.018	127	1.90
A	1.96	7.04	6.16	.012	67	1.00
L.S.D. at 5-percent level	1.23		.75	.007	32	

DEET						
Series 1b:						
A	0. 49	2. 12	0. 98	0. 006	202	1. 00
B	1. 17	2. 07	1. 09	. 004	232	1. 15
C	. 17	2. 18	1. 10	. 010	105	. 52
L.S.D. at 5-percent level	. 49		. 51	. 0007	87	
Series 2b:						
G	1. 26	1. 29	. 61	. 007	127	2. 05
H	. 88	1. 30	. 42	. 004	242	3. 90
A	. 73	1. 46	. 88	. 007	62	1. 00
L.S.D. at 5-percent level	. 35		. 21	. 002	59	
ETHYL HEXANEDIOL						
Series 1c:						
A	0. 91	2. 08	0. 88	0. 006	192	1. 00
B	2. 33	1. 95	. 91	. 006	200	1. 04
C	. 21	2. 06	1. 16	. 011	85	. 44
L.S.D. at 5-percent level	. 56		. 23	. 003	44	
Series 2c:						
G	3. 01	6. 54	2. 31	. 015	320	. 78
H	2. 72	6. 78	2. 17	. 011	380	. 92
A	1. 29	6. 80	3. 16	. 010	412	1. 00
L.S.D. at 5-percent level	1. 82		. 66	. 002	87	

Minimum Effective Dosage

The minimum effective dosage (MED) of three repellents on each of five subjects was determined in two series of tests, the first with subjects A, B, and C and the second with subjects A, G, and H. The MED will vary with the avidity of the mosquitoes, but a series of tests with mosquitoes of uniform avidity provides a valid comparison between different repellents and different individuals. In these tests a measured area of forearm was treated with various dosages of each repellent. The arms were exposed to mosquitoes 5 minutes after treatment and at half-hour intervals thereafter until a confirmed bite was received. The arms were rinsed with 500 ml. of distilled alcohol immediately after termination of the test, and the amount of repellent recovered was determined by spectrophotometry.

If no variations occurred from test to test and the MED could be determined exactly, it would be the lowest dosage that would barely prevent bites in the initial test, and consequently it would always allow bites after 30 minutes, when some of the repellent had evaporated or been absorbed. Since variations do occur, the MED can be expected to permit bites in the initial exposure in some tests and to give protection after 30 minutes in others. The results of these tests are given in table 12.

The MED's for deet were rather uniform on all subjects in both series of tests. Those for ethyl hexanediol were always higher than those for deet. They were much higher in the second series than in the first series (about three times as high on subject A), but were fairly uniform on all subjects in any one series.

The MED's for dimethyl phthalate were much higher than those for deet or ethyl hexanediol, but were fairly uniform on all subjects in both series of tests except on subject C, who was unable to obtain initial protection with dimethyl phthalate in most tests in this series, even though it was applied to the point of runoff. This was not due entirely to the inordinate avidity of the mosquitoes.

A comparison between the MED's in these tests and the residual dosages at the time of biting in table 11 shows that although the MED's for dimethyl phthalate were higher than the residues in table 11, those for the other two repellents were equal to the residues or lower, indicating that the avidity was within the normal range.

Hair and Sebum

Studies were made to investigate the effect of hair and sebum on the relative attractiveness of subjects A, B, and C and on the protection obtained with repellents. The left arm of each subject was shaved smooth. The arms were examined before each test and shaved when necessary to remove the stubble. The amount of hair removed from the arms was as follows:

	Amount of hair removed (mg.)	
Subject	*Total*	*Per square inch*
A	433.3	6.95
B	254.5	3.63
C	393.4	5.30

Biting rates in round-robin tests were taken as described previously. The results are shown in table 13.

The unshaved arms remained in the same order of attractiveness as observed previously, with subject B about three times as attractive as A and about four times as attractive as C. With the hair removed the attractiveness of all subjects was about equal, indicating that the thicker hair of subjects A and C gave more natural protection than the hair of subject B.

Tests were conducted to determine the rates of evaporation and absorption of three repellents from the shaved and unshaved arms of the subjects. The two arms of each subject were treated with the same repellent and exposed for 2 hours in the glass percolators. The results are given in table 14.

The evaporation rates were about the same on the shaved and unshaved arms of each subject with each repellent, except with ethyl hexanediol on subjects B and C, where the rate was slightly higher on the unshaved arms. The absorption rates showed greater differences than the evaporation rates between shaved and unshaved arms (up to 200 percent), but there was no consistent difference attributable to shaving. The absorption rate was higher on the shaved arm of A with deet and lower with the other repellents, whereas the reverse occurred with B and C.

The effect of hair on the protection time obtained with repellents and on the rate at which the repellents were lost from the skin was studied by means of paired tests with repellents applied to one shaved and one unshaved arm of each of three subjects. The results are given in table 15. The rates of loss were generally equal or about equal on shaved and unshaved arms, but when differences did occur the greater loss was on the shaved arm. With dimethyl phthalate on subject C the difference was substantial. Differences between protection periods on shaved and unshaved arms were greater but less consistent. The longer periods sometimes occurred on the shaved arms and sometimes on the unshaved. The amount of dimethyl phthalate required to give protection on subject C was much higher than in most previous tests, but it was lower than in the tests to determine the MED (table 12).

Tests were conducted to determine whether the sebum on the arm contributed to its attractiveness. The arms of two subjects were submerged to the elbow in 4 liters of purified acetone for 3 minutes every other day for 10 days. After the acetone was evaporated, the extracted sebum and particles of skin were left. The skin particles were removed by filtering. All but a trace of the sebum was soluble in reagent-grade chloroform, an indication of the absence of any proteinaceous material. The sebum recovered from subject B, who had the highest natural attractancy of the three subjects in previous tests, was 3.93 mg. per 100 square inches of skin per day and that from subject C, the least attractive, was 2.73 mg.

A series of tests was run to compare the biting rates on the arms of the subjects when one arm was rinsed by submersion in acetone for 3 minutes to remove the sebum and 1 ml. of acetone was spread on the other arm to serve as a check. The opposite arm of each subject was tested against each other and against each arm of the other subject. The arms were allowed to remain in the cage for only 20 seconds so that the counts would be low and the mosquitoes would not have time to become fully fed, but the counts were recorded in bites per minute.

TABLE 12.—*Time required for confirmed bites by* Aedes aegypti *with various dosages of 3 repellents and minimum effective dosage (MED) on 5 human subjects in 2 test series*

SERIES I

Subject	Dimethyl phthalate		Deet		Ethyl hexanediol	
	Dosage per square inch	Time to confirmed bite	Dosage per square inch	Time to confirmed bite	Dosage per square inch	Time to confirmed bite
	Mg.	*Minutes*	*Mg.*	*Minutes*	*Mg.*	*Minutes*
A	6. 36	0	0. 36	30	0. 55	0
	7. 59	0	. 36	30	. 57	0
	8. 47	0	. 38	30	. 57	60
	8. 74	30	. 39	65	. 66	65
	8. 74	60	. 49	95	. 71	95
	9. 03	30	. 56	65	. 74	65
	-----	-----	. 57	30	-----	-----
MED	8. 50	-----	<. 36	-----	. 57	-----
B	6. 34	0	. 30	0	. 62	35
	7. 27	0	. 34	30	. 63	0
	7. 43	0	. 35	0	. 77	0
	7. 46	30	. 36	30	. 77	0
	7. 92	30	. 38	30	. 88	60
	8. 06	90	. 47	75	-----	-----
	8. 73	120	. 52	95	-----	-----
	-----	-----	. 52	95	-----	-----
MED	7. 40	-----	. 35	-----	. 80	-----
C	12. 43	0	. 33	0	. 56	0
	13. 66	0	. 35	0	. 59	30
	14. 54	30	. 36	60	. 65	0
	18. 67	0	. 45	30	. 70	30
	22. 58	0	. 46	30	. 80	95

	----------	----------	.54	65	----------	----------
	----------	----------	.54	95	----------	----------
MED	>22	----------	.35	----------	.65	----------
SERIES 2						
A	7.03	0	0.32	0	1.41	0
	7.20	0	.32	0	1.47	0
	7.93	0	.32	30	1.54	30
	8.00	30	.38	30	1.70	30
	9.33	30	.39	30	1.93	60
	----------	----------	.46	30	----------	----------
	----------	----------	.57	30	----------	----------
	----------	----------	.68	60	----------	----------
MED	8.00	----------	.32	----------	1.50	----------
G	7.41	0	.41	0	1.41	0
	7.42	0	.49	30	1.66	0
	8.14	0	.50	0	1.76	0
	8.14	30	.51	30	1.83	30
	9.16	30	.59	60	2.04	30
	10.47	60	.63	60	2.31	60
	----------	----------	.64	90	----------	----------
	----------	----------	.65	90	----------	----------
MED	8.14	----------	.50	----------	1.80	----------
H	7.09	0	.25	0	1.38	0
	7.54	0	.26	0	1.43	0
	8.30	30	.28	0	1.46	30
	8.37	30	.36	30	1.74	60
	8.33	60	.37	30	1.88	60
	8.86	60	.44	30	----------	----------
	----------	----------	.49	60	----------	----------
	----------	----------	.54	90	----------	----------
MED	8.15	----------	.30	----------	1.45	----------

TABLE 13.—*Biting rate of* Aedes aegypti *on shaved and unshaved arms of 3 subjects*

Subject	Arm	Bites per square inch per minute	
		Range	Average
		Number	*Number*
A	Shaved	0.33–1.19	0.76
	Unshaved	.03– .25	.15
B	Shaved	.50–1.29	.77
	Unshaved	.25– .70	.49
C	Shaved	.50–1.44	.82
	Unshaved	.03– .27	.11
L.S.D. at—			
5-percent level			.13
1-percent level			.18

In each test the arms were exposed twice and an average count was taken. The biting rates in six replicate tests are given in table 16.

These results give some indication, not borne out by subsequent experiments, that removal of the sebum by rinsing decreased the attractiveness of the arms. The rinsed arms of B and C received less bites than the check arms, but the difference, though marked, was not statistically significant at the 5-percent level. As in previous tests, B's check arm received significantly more bites than C's check arm. However, when the arms of both subjects were rinsed, part of the difference was lost, and the bites received on B's rinsed arm were significant at the 7-percent level. There was no difference between the rinsed arm of B and the check arm of C. As expected, B's check arm received significantly more bites than C's rinsed arm.

Another series of tests was run to observe the effect of adding the extracted sebum to the rinsed arms. Exposures were made as in the preceding series. The two arms of each subject were first tested simultaneously without treatment to assure that no significant differences between the arms occurred. Then one arm was rinsed by submersion for 3 minutes in acetone, dried, allowed to warm to normal temperature, and tested against the untreated arm. The rinsed arm was then treated with sebum from the same subject, applied at the rate at which it had been extracted, and again tested against the untreated arm. Finally, the treated arm was again rinsed in acetone, dried, warmed, treated with sebum from the other subject, and tested against the untreated arm. The results are given in table 17.

In this series of tests, rinsing the arms to remove the sebum did not decrease the number of bites received, and adding sebum did not increase the biting rate. In fact, the only significant difference was a lowered biting rate on the arm of subject C, the less attractive subject, treated with sebum from B, the more attractive. It was concluded that the sebum had not contributed materially to the attractiveness of the subjects. However, it is possible that sebum contains some attractant that was lost during the evaporation of the acetone.

TABLE 14.—*Evaporation and absorption of 3 repellents from shaved and unshaved arms of 3 subjects during 2 hours in glass percolators. (2 tests)*

Repellent and subject	Amount of repellent per square inch					
	Applied to—		Evaporated from—		Absorbed by—	
	Shaved arm	Unshaved arm	Shaved arm	Unshaved arm	Shaved arm	Unshaved arm
Dimethyl phthalate:	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
A	6.94	6.93	0.53	0.45	0.09	0.23
B	6.69	6.47	.63	.58	.30	.21
C	6.57	6.28	.60	.56	.38	.22
Average			.59 ± .08	.53 ± .09	.26 ± .06	.22 ± .08
Deet:						
A	6.74	6.76	.32	.33	.69	.55
B	6.36	6.24	.33	.31	.20	.40
C	6.35	6.29	.32	.32	.28	.48
Average			.33 ± .01	.33 ± .01	.39 ± .10	.48 ± .06
Ethyl hexanediol:						
A	6.52	6.50	.56	.54	.14	.29
B	6.81	6.19	.46	.70	.20	.17
C	6.37	6.16	.38	.53	.38	.22
Average			.47 ± .05	.59 ± .05	.24 ± .07	.23 ± .06

TABLE 15.—*Protection time with 3 repellents on shaved and unshaved arms of 3 subjects against* Aedes aegypti *and rate of loss of repellents from arms. (2 tests)*

DIMETHYL PHTHALATE

Subject and arm	Amount of repellent per square inch			Protection time
	Applied	Recovered	Lost per minute	
	Mg.	*Mg.*	*Mg.*	*Minutes*
A:				
Shaved	7.13	5.30	0.011	169
Unshaved	6.91	5.88	.009	112
B:				
Shaved	6.72	4.38	.015	160
Unshaved	6.42	4.37	.013	155
C:				
Shaved	15.63	10.49	.059	87
Unshaved	14.92	9.06	.033	175
DEET				
A:				
Shaved	1.11	0.34	0.003	271
Unshaved	1.13	.45	.003	242
B:				
Shaved	1.15	.41	.004	210
Unshaved	1.12	.61	.002	225
C:				
Shaved	2.14	1.57	.005	104
Unshaved	2.00	1.00	.004	255
ETHYL HEXANEDIOL				
A:				
Shaved	2.10	1.32	0.005	168
Unshaved	2.26	1.56	.005	150
B:				
Shaved	2.16	.76	.005	262
Unshaved	2.05	1.07	.005	202
C:				
Shaved	2.00	1.41	.005	110
Unshaved	1.97	1.34	.005	130

TABLE 16.—*Biting rate of* Aedes aegypti *on arms without sebum (rinsed) and with sebum (check) of 2 subjects*

Subject and paired arms	Bites per minute	
	Range	Average [1]
	Number	*Number*
B, rinsed	21-115	65.2 ± 14.0
B, check	42-174	107.0 ± 19.9
C, rinsed	6-105	49.5 ± 13.5
C, check	15-174	87.5 ± 20.1
B, check	111-246	152.5 ± 20.4*
C, check	60-177	107.0 ± 17.6
B, rinsed	54-264	138.0 ± 28.2**
C, rinsed	60-126	82.5 ± 14.6
B, rinsed	60-135	95.0 ± 12.0
C, check	12-198	88.0 ± 23.4
B, check	39-258	162.0 ± 34.3*
C, rinsed	33-180	96.3 ± 21.3

[1] *Significant at 5-percent level; **significant at 7-percent level.

TABLE 17.—*Biting rate of* Aedes aegypti *on opposite arms of subjects B and C when one arm was untreated and the other was (1) untreated, (2) rinsed in acetone to remove the sebum, or (3) rinsed and re-treated with sebum from the same subject or the other subject. (8 tests)*

Paired arms	Bites per minute received by—			
	Subject B		Subject C	
	Range	Average	Range	Average [1]
	Number	*Number*	*Number*	*Number*
Untreated	33-330	143 ± 36	24-162	73 ± 7
Do.	48-222	147 ± 33	18-132	68 ± 12
Do.	21-309	119 ± 31	15-96	55 ± 12
Rinsed	27-357	180 ± 37	18-93	52 ± 9
Untreated	21-132	84 ± 14	12-102	57 ± 12
Sebum from B	21-207	90 ± 23	12-21	19 ± 1*
Untreated	33-156	100 ± 17	15-216	70 ± 28
Sebum from C	20-168	77 ± 18	9-63	31 ± 6

[1] *Significant at 5-percent level.

EFFECTIVENESS OF REPELLENTS ON HUMAN AND ANIMAL SKIN

The relative initial effectiveness of three repellents on the skin of humans and animals was determined by means of the minimum effective dosage (MED). In this experiment effectiveness was based on protection at the level of five bites in a 3-minute exposure rather than a confirmed bite. The hair was clipped from the sides of a steer and rabbit and the belly of a pig and guinea pig. Several dosages of each repellent were applied from a pipette to the clipped areas of the animals and to the unshaved arms of a man and a woman, subjects A and G. The treated areas in square inches were 16 on the steer and pig, 7.5 on the rabbit and guinea pig, 63 on the man's arm, and 54 on the woman's. Within 5 minutes after treatment the treated area was exposed to mosquitoes.

In these tests the 7- to 8-day-old mosquitoes were confined in cylindrical wire cages 2½ inches in diameter. There were from 41 to 111 mosquitoes per cage, with an average of 69. During testing, the cover was removed from one end of the cage, and the open end was held against the treated skin for 3 minutes, as shown in figure 5. If less than five bites were received, exposures were made each half-hour until five bites did occur. The results are shown in table 18.

The MED for dimethyl phthalate was about the same (0.07 to 0.10 mg. per square inch) on all hosts except the steer, where it was about double (0.17 mg.).

FIGURE 5.—Testing the effectiveness of a repellent applied to a young pig. The cylindrical cage contains *Aedes aegypti* mosquitoes. It is open at the lower end, so that the mosquitoes must stand on the treated skin to bite.

TABLE 18.—*Minimum effective dosage (MED) of 3 repellents on various hosts against* Aedes aegypti

Repellent and host	MED of repellent per square inch directly after treatment and 30 minutes later [1]				
	0 minutes		30 minutes		
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Dimethyl phthalate:					
A	0.07	0.09	0.09	0.11	0.12
G	.07	.08	.10	.11	.13
Steer	.15	.15	.19	.21	.25
Guinea pig	.06	.07	.07	.10	
Rabbit	.07	.07	.11	.14	
Pig	.09	.09	.12	.13	.19
Deet:					
A	.15	.15	.16	.17	.20
G	.07	.07	.09	.09	
Steer	.26	.32	.42	.61	
Guinea pig	.07	.07	.07	.10	.14
Rabbit	.07	.07	.10	.11	.14
Pig	.06	.06	.10	.11	
Ethyl hexanediol:					
A	.10	.12	.12	.17	.17
G	.10	.10	.13	.13	.19
Steer	.48	.50	.49	.50	.56
Guinea pig	.08	.09	.10	.13	
Rabbit	.06	.07	.09	.09	
Pig	.10	.20	.19	.20	.21

[1] Based on protection at level of 5 bites in 3-minute exposure.

The MED for deet was about 0.07 to 0.09 mg. per square inch on subject G, guinea pig, rabbit, and pig. On subject A it was about double this amount (0.15 mg.), and on the steer it was about double that on subject A (0.37 mg.).

The MED for ethyl hexanediol ranged from 0.08 to 0.12 mg. per square inch on subjects A and G, guinea pig, and rabbit. It was about twice as high on the pig (0.20 mg.) and four times as high on the steer (0.50 mg.).

The residual effectiveness of the repellents on the various skin surfaces, as affected by their loss by evaporation and absorption, was also studied. A dosage of about 0.5 mg. per square inch *above the MED* was applied to each skin surface. The MED should give initial protection, allowing bites at 30 minutes, and the extra 0.5 mg. should extend the protection period beyond 30 minutes. Differences in the protection period would thus be due solely to differences in the rate of loss of the additional material and would not be affected by differences in the relative effectiveness on the different hosts. The results are given in table 19.

The steer lost all three repellents faster than any of the other hosts, as shown by the shorter protection time. There were no consistent differences between either of the human subjects and the guinea pig, pig, and rabbit, but subject A always had shorter protection periods than G.

Under the standardized conditions of these tests the repellents were about equally effective on human subjects, guinea pig, pig, and rabbit,

TABLE 19.—*Effectiveness of 3 repellents on various hosts against* Aedes aegypti *at dosages of 0.5 mg. per square inch above the MED.* (*4 tests*)

Repellent and host	Intended dosage per square inch	Dosage applied per square inch		Protection time	
		Range	Average	Range	Average
	Mg.	*Mg.*	*Mg.*	*Minutes*	*Minutes*
Dimethyl phthalate:					
A	0.59	0.52–0.60	0.56	60–150	120
G	.59	.59– .60	.60	122–180	152
Steer	.67	.66– .68	.67	45–60	52
Guinea pig	.57	.59– .60	.59	90–90	90
Rabbit	.59	.63– .63	.63	90–90	90
Pig	.60	.63– .63	.63	100–135	117
Deet:					
A	.65	.59– .66	.63	84–149	112
G	.58	.50– .65	.58	165–189	183
Steer	.87	.80– .82	.81	60–90	81
Guinea pig	.57	.62– .63	.62	210–210	210
Rabbit	.59	.57– .62	.60	175–205	192
Pig	.58	.57– .61	.57	210–240	223
Ethyl hexanediol:					
A	.62	.60– .62	.61	60–120	90
G	.62	.63– .65	.64	207–210	209
Steer	1.00	.92– .95	.93	60–60	60
Guinea pig	.59	.59– .59	.59	60–120	90
Rabbit	.58	.58– .59	.59	120–120	120
Pig	.70	.69– .73	.71	198–240	220

but less effective on the steer. The lower effectiveness on the steer was due in part to the higher MED and in part to the greater rate of loss. However, the higher MED was itself probably required by the obviously greater absorptivity of the skin, which also caused the more rapid loss. The rapid absorption of the repellent by the skin was visually evident at the time of application.

SUMMARY

The protection period conveyed by any given dosage of repellent depends on (1) the minimum effective dosage, or MED, which is the minimum amount per unit of surface required to protect against the given population of insects, and (2) the rate of loss, or rate at which the applied dosage is depleted to the level of the MED.

The principal mode of loss of repellents in practical use is usually abrasion, caused by rubbing the treated surface of the skin against the clothing or other objects, and this will vary with the activity of the subject. Under experimental conditions, when loss by abrasion was prevented, subjects lost repellent by evaporation and absorption. Under uniform conditions of temperature, humidity, and air circulation, evaporation rates were usually about the same on different men and women, but absorption rates showed more variation and probably account for most of the individual differences observed in the total rates of loss. Evaporation rates were about twice as high from human skin and three times as high from guinea pigs as from cloth. Di-

methyl phthalate and ethyl hexanediol evaporated about twice as fast as deet but were absorbed more slowly. The rate of evaporation in milligrams per square inch remained relatively constant through a wide range of dosages above a critical minimum level.

Deterioration of the repellent on the skin, which has also been postulated as a possible mode of loss, did not occur, and repellents did not lose effectiveness on the skin by admixture with emanations from the body, such as sweat and carbon dioxide. The amount of deet remaining on the skin for 30 to 180 minutes after treatment was as effective as the same amount of deet freshly applied.

In extensive tests with three men and two women, one male subject, subject C, consistently showed the shortest protection time, particularly in tests with dimethyl phthalate. This was associated in part with a higher MED for dimethyl phthalate on this subject, but more frequently with a more rapid rate of loss. Subject C was the least attractive of the subjects, and his arms gave off the most water and the least carbon dioxide, whereas subject B, the most attractive, gave off the least water and the most carbon dioxide. The amount of hair on the arms appeared to be inversely correlated with the biting rate of the mosquitoes on untreated arms, but it had no consistent effect on the protection periods obtained with repellents. Studies on the effect of sebum were inconclusive. There were no consistent differences between the men and the women subjects in evaporation, absorption, attractiveness, or protection time.

The MED with any given repellent against mosquitoes of uniform avidity varied only slightly between four of the five subjects. The MED for ethyl hexanediol was 2 to 5 times as high as that for deet, and that for dimethyl phthalate was about 20 times as high.

The relative initial effectiveness of dimethyl phthalate, deet, and ethyl hexanediol, as determined by means of the MED's, was about the same on the skin of humans and the shaved skin of a pig, a rabbit, and a guinea pig, but on a steer the MED's were two to six times as high as on the other hosts. The steer also lost all three repellents faster than the other hosts.

Differences in protection periods between repellents appear to be due principally to differences in the MED, and secondarily to differences in the rate of loss, where resistance to loss by abrasion would be an important factor. Differences in protection periods between individuals appear to be due primarily to differences in rate of loss, principally by absorption if loss by abrasion is uniform, and to a lesser extent to differences in the MED. Relative individual attractiveness without repellent is not, apparently, correlated with the individual MED.

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U.S. GOVERNMENT PRINTING OFFICE 1963

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