CHAPTER IX

Sandfly Fever
(Pappataci, Phlebotomus, Three-Day Fever)

Marshall Hertig, Ph. D., and Albert B. Sabin, M.D.

Part I. History of Incidence, Prevention, and Control

Marshall Hertig, Ph. D.

GENERAL CONSIDERATIONS

Sandfly fever is a short-term febrile disease of virus etiology transmitted by the bite of one or more species of sandflies of the genus Phlebotomus.\(^1\) Although there are no fatalities and the victims are incapacitated usually for no more than 1 or 2 weeks, the disease is potentially of great military importance. This is because large numbers of men may be incapacitated at precisely the time when they are most needed. The danger would be especially great for invading forces which had not been previously exposed to the disease and would therefore be composed of nonimmune persons. The defending forces, whether composed of natives or troops who had been in the regions during the previous sandfly season, would be mostly immune. Since the incubation period is very short, a matter of only 3 to 6 days, it would be possible for the invaders to have a large fraction of their forces rendered non-effective in the first critical days of a campaign.

Military History

Since armies, rather than resident populations, have been the chief, or at least the most spectacular and articulate, sufferers from sandfly fever, its history is very largely military. The classic investigations in Dalmatia on the etiology and transmission were made by an Austrian military commission consisting of Doerr, Franz, and Taussig.\(^2\) Most of the later experimental work on the virus and its transmission has been performed under military auspices. Some of the first studies on the bionomics of Phlebotomus, carried out by various

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\(^1\) War Department Technical Bulletin (TB MED) 82, 8 Aug. 1944.


British investigators in Malta before World War I, were stimulated by the severe problems of sandfly fever and sandfly annoyance in local military establishments. A large proportion of our epidemiologic information has come from experiences of British forces at military stations and during campaigns in the Mediterranean region, the Middle East, and in India. Americans in general had no experience with the disease before World War II.

The military importance of the disease in all cases has rested on the central fact that during the sandfly season newcomers could suddenly be rendered non-effective in great numbers. This has applied not only to newly arrived foreign troops but also to natives who came from regions where the disease did not occur. For example, Indian troops from certain hill regions have been as severely affected as the British. The military effects have ranged from interference with routine garrison duties and training schedules and temporary overcrowding of hospitals to ineffectiveness in combat. Sinton gave an instance from the Waziristan campaign in 1917 where a battalion was attacked almost en masse by sandfly fever and rendered temporarily unfit for further service. He cited the reports of others that the disease was the cause of “a great deal of ineffectiveness among troops of the Mesopotamian Expeditionary Force” in World War I, and that it accounted for 50 percent of the cases of sickness among overseas personnel of the Royal Air Force. In Palestine during World War II, the operation of air-training schools with very crowded schedules was seriously affected by the disease. The experience of the U.S. Army in World War II in which there were an estimated 8,500 cases of sandfly fever in the Sicily Campaign and potentially serious outbreaks on the Italian mainland is reported by Sabin (pp. 168-174).

Epidemiology

Distribution.—Sandfly fever is limited chiefly to the Old World between latitudes 20° and 45° north. It extends from the Mediterranean to India and Burma and possibly to China. The following details as to distribution are quoted from Sabin, Philip, and Paul:

The disease is definitely known to occur in Italy as far north as the Po Valley, Sicily, along the Adriatic coast of Yugoslavia as far north as the Istrian peninsula, Greece.


5 See footnote 4(1).


Malta, Crete, Cyprus, Egypt, Palestine, Syria, Iraq, Persia (Iran), Crimea and the Transcaucasian region, and the northwest and central provinces of India. There are also reports which would suggest that this disease may occur in China as far north as Peiping and Tientsin and as far south as Hong Kong, in Burma along the coast of the Bay of Bengal, in Ceylon, in the Poona region of India, in Aden and along the adjacent Red Sea coast of Arabia, the Anglo-Egyptian Sudan, along the Mediterranean coast of Africa—particularly the eastern portion, Corsica, the Mediterranean coast of France, Gibraltar, and along the Atlantic coast of Portugal. Reports of a similar disease in countries lying just north or south of the Equator have come from Kenya and the Tanganyika Territory in Africa and from the region of Bolivar [Colombia] in South America. The disease is not known to occur in the United States.

In Panama, there occurs sporadically a short-term fever which is clinically similar to sandfly fever and which has at times been diagnosed as such. However, the identity of this disease with sandfly fever has never been established nor, so far as known, has any experimental work with Phlebotomus and sandfly fever ever been undertaken in the Western Hemisphere. It may be remarked that in Panama there are a number of species of Phlebotomus which bite man. Their distribution is sufficiently wide and varied as to habitats to permit their consideration as vectors.

In the Pacific areas, cases of sandfly fever were reported but never confirmed by later investigation, as shown by Lt. Col. (later Col.) Cornelius B. Philip, S.C. (pp. 123-124). Sera from a number of cases were shipped to the United States and inoculated into volunteers. Sera from the Philippines gave only negative results, while from the New Guinea specimens four strains of typical dengue were recovered.

The occurrence of sandfly fever in the Western Hemisphere and in the Pacific remains unproved.

**Symptomatology and diagnosis.**—Clinically, the disease is characterized by sudden onset, fever lasting usually about 3 days (whence the name 3-day fever) ranging from 100° to 105° F., with severe frontal headache, pain in the eyes, photophobia, pain in the back and joints, and general malaise. The disease may simulate influenza, other respiratory infections, the initial stages of other febrile diseases, and may be confused with dengue. From the latter, it may usually be distinguished by the absence of a rash and the shorter febrile period.

A decrease in the number of segmented neutrophiles together with a relative and absolute increase of immature neutrophiles is a phenomenon shown by Sabin and others (p. 110) to be constant in sandfly fever and an important aid in diagnosis.

There is no distinctive sign or specific test, and, as a result, diagnosis is often difficult, particularly under field conditions. The practical implications are illustrated by the Army’s experience in Sicily where many undiagnosed febrile cases, which were undoubtedly sandfly fever, were treated as malaria and needlessly evacuated to North Africa (pp. 168-174).

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Immunity.—Native populations are seldom affected severely by sandfly fever. Indeed, the people may hardly be aware that there is such a distinct disease entity, even in an area capable of producing an epidemic among newcomers. This was the case in Naples in 1944 (p. 121). The scattered cases which were found among civilians by systematic search had been attributed to colds and the like and had not been deemed worthy of medical attention. Infection is obviously acquired during childhood, with a considerable degree of resulting immunity which is renewed from time to time by subsequent infections. From the readiness with which newcomers may contract the disease in the absence of any recognized outbreak among the local population, it is evident that opportunities for reinfection of the latter are a more or less constant feature of the epidemiology.

The duration of the immunity in an adult from a single attack has been shown experimentally by Sabin and others to be at least 1 months and even as long as 2 years (p. 148), but that it may have disappeared after 4 to 7 years. In any case, it appears that the immunity from a single attack and from whatever reinfections may occur naturally is such that troops who remain in the same area are, like the local civilians, little troubled by sandfly fever. However, reports of second attacks in the same season nearly always have been associated with a change of station. A reasonable explanation for such repeated attacks is provided by the demonstration that there are at least two immunologically distinct strains in the Mediterranean area; namely, the Sicilian Middle East strain recovered in 1943, and the Naples strain recovered in 1944 (pp. 165-166).

Criteria for the identification of the virus.—Sabin has pointed out that the ultimate criterion for the identification of a given strain of virus, as that of sandfly fever, is the actual demonstration of its transmissibility by Phlebotomus (p. 152). This was done in the case of the Middle East strain. A procedure of this sort is possible only under certain special conditions. It was not feasible in the case of the Naples virus, but there remains no reasonable doubt that it was sandfly fever. Clinically and epidemiologically, it followed the classic pattern, and it was amply shown by competent observers to be associated with Phlebotomus papatasi. In practice, in the face of an outbreak of what appeared to be sandfly fever, control measures would, of course, be instituted as soon as possible. There would be neither necessity nor justification to await the definitive demonstration of transmission by Phlebotomus.

The following seem to be reasonable grounds for assuming a given outbreak to be sandfly fever and for proceeding on that assumption with whatever control measures are feasible:

1. Correspondence with symptomatology and clinical course of sandfly fever.
2. Occurrence during the sandfly season in a known endemic area.
3. Occurrence in newcomers, with the local population apparently unaffected.
4. Demonstrated association with *Phlebotomus*, especially *P. papatasii* within its range: Sandflies found in fair abundance either in the act of biting at night or at rest in living quarters during the day. It would usually be possible to demonstrate the converse; namely, that specific quarters, barracks, or localities with little or no disease had few or no sandflies, or at least that *P. papatasii* was scarce or absent.

5. Prompt cessation of the outbreak on the application of control measures, such as residual DDT (p. 121).

**Etiology and Transmission**

Duerr and others (p. 109) working on the Dalmatian coast in 1908, showed that the etiologic agent was filterable, that blood taken from patients on the first day could produce the disease when inoculated into other persons, and that the disease could be transmitted by *P. papatasii*. These basic facts have been confirmed by a number of later investigations in the Mediterranean, India, and Transcaucasia. No insect other than *Phlebotomus* has been shown capable of transmitting the infection, and no animal other than man is known to be susceptible.

**Insect vector.** — *P. papatasii* is the species with which all recorded experimental infections have been accomplished and is the species which has been found associated with the disease in those areas where sandfly fever has been most studied. *P. papatasii* is one of the most widely distributed species of the genus and occurs in an extraordinary variety of habitats from the Mediterranean to the western half of India (fig. 4). Around the Mediterranean littoral, it is found, often in great abundance, in rural areas as well as in the heart of large cities, such as Naples and Athens. In the salt desert around the Dead Sea, this species occurs in enormous numbers even at some distance from human habitations. In certain semidesert regions of Turkestan, it has been found breeding in the burrows of rodents. The possibility that other species of *Phlebotomus* may also be vectors has not been investigated experimentally. Outside the range of the *P. papatasii*, for example, in Burma and China, if the reported disease is actually sandfly fever, other species must necessarily be involved.

**Life history.** — The breeding places of *P. papatasii* are in moist, loose soil in dark, humid, sheltered places such as those beneath stones, in masonry crevices, in deep soil cracks, or in animal burrows. The female secures a blood meal and after several days lays a batch of eggs, a process which may be repeated several times. The males do not suck blood. The eggs hatch in about 10 days. The larvae are scavengers and feed on insect or animal feces, decaying

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vegetation, or other organic debris. The larval development usually takes from 2 to 4 weeks, and the pupal stage requires another week or 10 days. The whole life cycle normally takes at least 6 to 8 weeks. Overwintering occurs in the last (fourth) larval stage. The sandflies begin to emerge about April and have largely disappeared by October or November. There is no evidence that adults can overwinter.

**Habits and flight range.**—The adult sandflies become active about dusk and may feed at any time during the night, seeking shelter by morning. The flight is silent, which is reflected in the name *papatasii*, a word of Italian derivation said to mean "bite, say nothing." They are able to pass easily through ordinary screening or mosquito netting. Contrary to repeated statements, they may be found on the upper floors of buildings, from 50 to 70 feet above ground level. *P. papatasii* tends to remain in rooms where it has fed and may often be found in great numbers in the upper corners.

The flight range of *P. papatasii*, in common with that of sandflies in general, is very short, usually a matter of only 100 meters or thereabouts from their breeding places. This at times leads to extreme local spottedness of distribution, so that certain sections of town, or groups of houses, or even certain rooms may be heavily infested while there are few sandflies in the immediate surroundings. The peculiarities of local distribution depend on the availability of potential breeding places and of daytime shelters, and to a certain extent on the prevailing winds and local air currents. An exception to the normally
short flight range has been noted in desert habitats, where with marked sandflies a range of 1,500 meters has been measured.

Sandflies have often been kept alive in the laboratory for several weeks, and it is supposed that this reflects their longevity in nature. With suitable technique, they can be fed repeatedly in the laboratory, and it is known (from the state of the ovaries and the glands) that they commonly refed at least once in nature, a point of importance in the transmission of a disease from person to person.

Phlebotomus and the virus.—The experimental evidence indicates that sandflies may become infective about a week after they have fed on a patient, but the extremes of time required for the development of the virus have not been determined. Throughout the sandfly season, there are continuous opportunities for the transmission of the virus from person to person. Carrying the virus over the winter, however, has been more difficult to explain. The virus is circulated in the blood of man for only 2 or 3 days, and no other vertebrate host is known. Early in the study of the disease, it was suggested that the sandfly larvae might become infected either via the egg or by eating the dead bodies of sandfly adults and thus carry the virus through the winter. Certain investigators have succeeded in transmitting the disease by the progeny of infected sandflies, while Sabin and others have failed.

In this connection, there occurs to the writer a point which he has not seen discussed; namely, that the experimental infection of sandfly progeny via the larvae may happen more readily with a generation which is actually destined to overwinter. P. papatasii, like other species of Phlebotomus in climates with a cold winter, exhibits the phenomenon of the diapause; that is, larvae from eggs laid toward the end of the sandfly season develop normally to the fourth instar but do not pupate until the following spring. This phenomenon is not the response of the individual sandfly larvae to lowered temperature or other external factors but is characteristic of that particular generation. In the laboratory, larvae undergoing the diapause stubbornly persist in that state in spite of efforts to bring them out of it. Overwintering larvae should be compared with those of the summer generations as to their ability to harbor the virus. The negative translarval transmission experiments of Sabin and others (p. 110) were done with sandflies reared during the early part of the sandfly season. Corresponding data for the positive experiments were provided by Whittringham and by Moshkovsky and others who, however, do not indicate the time of year when their studies were made.

Whatever the factors are which permit the virus to be carried over to the next sandfly generation, it has been demonstrated that it can happen at least part of the time. This provides the most likely explanation of how the virus is carried over from season to season.

Control measures.—The first really effective control measure against Phlebotomus, namely, residual DDT, was developed and tested only during
World War II. Control measures, together with protective measures, are discussed later.

GEOGRAPHIC DISTRIBUTION

The principal areas where sandfly fever occurred in U.S. troops were the Middle East, China-Burma-India, and Mediterranean theaters. In general, the disease was limited to regions long known to be endemic, and outbreaks corresponded with the seasonal prevalence of sandflies (Phlebotomus). Whenever studies were made, association of the disease with sandflies was demonstrated. The incidence of sandfly fever in these three theaters is summarized in table 20.

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1 Includes Alaska and Ireland.
2 Includes North Africa.
3 Includes 41 cases on transports.

Note.—Absolute zero is indicated by zero in the units column; 0.0 indicates a rate of more than zero but less than 0.01 and 0.00 a rate of more than zero but less than 0.005.

Middle East Theater

Persian Gulf Command.—The Middle East theater had a higher rate of sandfly fever than any other theater. The Persian Gulf Command had a particularly high rate, about 50 percent more than that of the theater as a whole. The great majority of cases occurred between April and October, with peaks occurring from June to August, chiefly in August.11

There was a steady decline in the entire theater from a rate of 70.13 per 1,000 troops, per annum, in 1942 to 3.42 per 1,000 in 1945. Rates in the Persian

Gulf Command, for example, which showed a peak of 235 per 1,000 in August 1943, reached only 74.91 in June and 72.37 in August 1944. The annual rates for 1943 and 1944 in the Persian Gulf Command were 60.2 and 29.79, respectively. The decrease was probably due both to improved protective and control measures and to the steadily increasing proportion of immune persons.

**British Army.**—Sandfly fever was also a problem to the British Army Middle East Forces, with a rate of 21.48 per 1,000 per annum in 1942. This covered the areas of Egypt, Palestine, Syria, Cyprus, Sudan, Eritrea, Malta, and Aden. An outbreak in two hospitals in the Middle East (which the writer understands were in Palestine) was reported by Cullinan and Whittaker. In both hospitals, about one-fourth of the doctors and nurses and nearly all the "other ranks" were attacked. On a single day in one hospital, one-quarter of the total strength of about 350 was sick. Of 1,910 patients admitted during the 3-month period, August to October 1942, nearly one-fifth contracted sandfly fever after admission. The height of the epidemic was between 27 August and 10 September. Cases were limited chiefly to certain wards surrounded by rubble, while others with "tidy surroundings" had relatively few. Sandfly infestation was heavy. A notable feature was that second attacks occurred in 15 percent of the cases from 2 to 12 weeks after the first, with occasionally three separate attacks.

On a visit to Palestine in 1944, the writer was informed that the British had been hampered by sandfly fever in 1943–44 at various airfields, particularly in maintaining schedules at air-training schools (p. 110).

**Cairo.**—Cairo provided an illustration of the classic epidemiology of the disease. Although troops quartered in Cairo had a high sandfly fever rate during the summers of 1941, 1942, and 1943, the disease was hardly recognized among the native population. From verbal reports, members of the Commission on Neurotropic Virus Diseases, Army Epidemiological Board, gathered that the disease was thought to be one of childhood but that almost nothing was known of its prevalence among adult civilians. The difficulty of diagnosis and the ease with which the disease may be mistaken for influenza made it seem likely that many cases reported as influenza might actually have been sandfly fever. In this connection, the Commission cited the morbidity rates recorded in Egypt for 1938 and 1939 which showed the highest influenza frequency and the next to lowest mortality in the month of July.

American troops were quartered at the Metro Barracks near Heliopolis, surrounded by city buildings, vegetation, and trees. In spite of protective measures, Philip, Paul, and Sabin reported that the incidence of sandfly fever

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in this unit during the fall of 1943 was approximately 25 percent of the entire command. During the same summer, British troops at three urban camps, comparable in location and vegetation to the Metro Barracks, also had considerable sandfly fever. At one camp, Helmleq, the incidence among the personnel of one hospital was especially high, more than half the officers, nurses, and other ranks having contracted the disease.\textsuperscript{15} In contrast to the situation in Cairo itself was the experience of the American troops at Camp Russell B. Hucy.\textsuperscript{16} Hucy, which had been established in the desert about 12 miles from Cairo. There were no trees or other vegetation. Sandflies were rare, and sandfly fever was practically nonexistent.

\textbf{Vector species.}—\textit{Phlebotomus papatasi}, one of the most widely distributed species of this genus, is practically the only one which has been associated with sandfly fever and is the only proved vector; that is, the only one with which experimental transmission to man has been effected. The species is found throughout the Middle East and was the one shown to be associated with outbreaks in Palestine and Cairo. Information was lacking about the specific association of sandflies with the disease in other areas in this theater.

\textbf{China-Burma-India Theater}

In the China-Burma-India theater, sandfly fever was first reported in September 1942. After October, no further cases were reported until February 1943. The greatest incidence occurred from May to July, the hot, dry season. In 1943, several explosive outbreaks were confined to small areas. As high as 40 percent of the members of one command were attacked at one time.\textsuperscript{16} The peak in each year was in July, the rates for that month in 1943, 1944, and 1945 being, respectively, 95.00, 26.75, and 4.80 (6.34 for Burma-India) per 1,000 troops.\textsuperscript{17}

A noteworthy feature of sandfly fever in this theater was that its presence was demonstrated in areas not previously thought to be endemic. It had been generally stated to be limited to the northwestern part of India. However, a number of cases were reported from Calcutta and elsewhere in eastern India,\textsuperscript{18} and in Burma in the Upper Chindwin section, around Rangoon, in Mandalay, and along the Salween River.\textsuperscript{19} Nevertheless, U.S. troops in Assam and northern Burma were singularly free from sandfly fever, and it is questionable whether the disease occurred in these areas. A decrease in the reported rate per 1,000 per annum occurred from 1943 to 1945 in the China-Burma-India theater, as it did in the Middle East. General insect control measures, such


\textsuperscript{17} Field Medical Bulletin, Headquarters, Services of Supply, U.S. Army Forces, China-Burma-India Theater, vol. 2, No. 9, September 1943.


\textsuperscript{19} War Department Technical Bulletin (TB MED) 174, July 1943.
as the use of DDT, probably contributed to the lowered incidence during 1945. An additional factor was that during the summer of 1945 only a small part of the theater strength was stationed in or required to pass through areas where *P. papatasii* was present.

**Vector species.**—*Phlebotomus papatasii* is common in northwestern India but is not known to occur east of a line drawn roughly from Delhi to Madras, with the exception of an isolated record near Calcutta. Its association with sandfly fever in northwestern India has been frequently cited in the literature. Shortt and others transmitted the disease experimentally by the bites of *P. papatasii* fed on sandfly fever patients in Peshawar, Northwest Frontier Province. In the eastern half of India, a number of species are known to exist, and at least three species have been found in Burma. There is no information, however, as to specific vectors in the eastern areas.

**Mediterranean Theater**

The invasion of North Africa in November 1942 took place after the close of the sandfly season. No cases were reported until July 1943. A peak of admissions occurred in September when the rate was 8.02 per 1,000 troops per annum.

**Sicily.** In the Sicily Campaign during the summer of 1943, there was a combined total of 14,492 cases diagnosed as sandfly fever, F.U.O. (fever of undetermined origin), and malaria (p. 172). The Commission on Neutotropic Virus Diseases visited Sicily in the latter part of the campaign (pp. 168-174). It was apparent that a large proportion of the undiagnosed fevers, and also of those diagnosed as malaria without positive blood films, were probably sandfly fever. Indeed, of 922 cases of the same group of fevers which were carefully observed at one hospital in Sicily, 637 were sandfly fever, representing an 87.6-percent proportion of the total cases (727) of sandfly fever (637), F.U.O. (4), and unclassified malaria (negative smear, diagnosed clinically) (86). If the same proportion of sandfly fever which was found among this group of “fevers” is applied to the “fever” cases in the campaign, and the proportion held throughout, approximately 8,500 may have been sandfly fever. Most of the American medical officers had had no previous experience with sandfly fever, but after the first few weeks they began to recognize it more readily as a distinct entity. The serious features from the military standpoint were not only the loss of the services of so many men but the fact that many were treated as malaria cases and needlessly evacuated to North Africa. The Sicilian campaign afforded a striking illustration of the serious threat which sandfly fever poses for an invading army of nonimmune men in the critical period of establishing a beachhead.

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30 See footnote 913, p. 113.
31 See footnote 19, p. 118.
Epidemiologic and entomologic studies, Italy, 1944

The Army had further experience with sandfly fever on the Italian mainland in 1944. Early in August, Lt. Col. Ross L. Gauld, MC, 15th Medical General Laboratory, Naples, Italy began an epidemiologic study of the disease. The writer was on temporary duty in the Mediterranean area from the last week in July until December 1944 for the purpose of studying Phlebotomus in relation to sandfly fever. In carrying out these investigations, Colonel Gauld and the writer made a number of journeys to various parts of Italy south of the Arno River and to Palestine. The following discussion represents first-hand observations or information received in the course of this work, which were embodied in various reports, jointly or separately, to the surgeon of the North African theater.23

NAPLES-CASERTA OUTBREAK

In 1944, a few cases were reported in June. In the first few days of July, shortly after the transfer of Headquarters, North African Theater of Operations, U.S. Army, from Algiers to Italy, there was a series of sharp, local outbreaks of sandfly fever in the Naples-Caserta area. These outbreaks tended to be limited to certain buildings, in which as many as one-third or even more of the occupants were affected. Frequently, entire offices were seriously crippled at a critical time. For example, enlisted men of Headquarters were billeted in the huge cavalry barracks across the street from the “Palace” at Caserta. The men in this building (and in a similar one occupied by the British) were most affected. Relatively few cases occurred among the officers billeted in the town or in the “tent city” in the forest behind the Palace. Troops quartered within a half mile of the cavalry barracks, as, for example, a Women’s Army Corps detachment in the hospital area in the Palace grounds, had very few cases. In the heart of Naples, in certain schools used as barracks, personnel suffered severely for several weeks before control measures were carried out. A large number of Army and Navy personnel engaged in the planning of the invasion of southern France were quartered in the “blockhouse,” a huge building in the form of a hollow square, located on a hilltop in Naples. The work was seriously threatened at an extremely critical period by

an outbreak of sandfly fever. Fortunately, most of the cases were limited to one corner of the building (an illustration of the sharp localization of outbreaks as encountered in Italy). There were certain rooms with 20 or 30 men in which nearly every man contracted the disease.

**First use of DDT.**—After the first part of August 1944, by which time control measures, including the spraying of quarters with DDT, had been put into general practice, the number of sharp outbreaks decreased. That this decrease probably was not seasonal was indicated by an epidemic in one unit quartered in an apartment building in Naples about three blocks from the Peninsular Base Section headquarters. During August, when no control measures were being taken by this unit, there were 30 cases among approximately 100 officers and enlisted men. *P. papatasii* was found in the sleeping quarters which occupied the fourth floor, showing that sandflies can be found in significant numbers some distance (at least 50 feet) above ground level, contrary to repeated statements in the literature. After spraying with DDT, the sandflies disappeared and the sandfly fever promptly ceased.

**BARI AND “HEEL,” ROME, AND SOUTH OF ARNO RIVER**

In Italy, the Naples-Caserta area was by far the most severely affected. At several installations of the Twelfth Air Force in the “heel” southeast of Bari, there were scattered cases during July 1944, which caused some concern. A survey of the Rome area in mid-August revealed relatively few cases. However, in a school building used as a barracks by the British, an outbreak occurred similar to the ones experienced by American troops in Naples, but on a much smaller scale. In the Fifth U.S. Army area, a survey made during the first week in September, in the region south of the Arno River from Florence to Cecina, showed that there had been very little sandfly fever. Very few sandflies were found near military installations, which were mostly located in open country or woodland away from towns and villages.

**SANDFLY FEVER IN CIVILIANS POPULATION**

Little could be learned from Italian sources about the epidemiology of sandfly fever in Italy. No published reports were located at the time these surveys were made, although several such reports were subsequently found. Physicians in Naples and Rome had general or "textbook" information rather than specific knowledge about the disease in their own cities. The population of the endemic area seemed to be largely immune, and cases were limited to childhood infections or sporadic cases which may not have been recognized as a distinct clinical entity. In the Naples apartment house just mentioned where the disease occurred in a military unit, an investigation was made of civilian families occupying the same building. The survey was made early in Septem-
ber 1944 by Italian physicians “borrowed” for the purpose from one of the Allied agencies. They found several actual cases and obtained histories of others consistent with sandfly fever which had occurred during July and August.

The cases reported for the Mediterranean theater in 1944 (a total of 1,363 with a rate of 6.72 per 1,000 per annum) were mostly from Italy, and the total number which occurred was undoubtedly considerably greater than that reported. In the Caserta-Naples outbreak, many cases, particularly early in July, were reported as F.C.O., even though many medical officers recognized at the time that they were probably dealing with sandfly fever. The diagnosis of sandfly fever was made more freely in the latter part of the summer.

GREECE

The experience of the Germans in the Mediterranean region was apparently comparable to that of the Allies, though there are few data available. Hallmann 25 reported that on the Greek mainland in the Athens district and on the islands about 20 percent of the German troops had sandfly fever in July and August 1941.

The writer, while engaged in work on Phlebotomus and DDT in Greece in 1948, 26 learned that the Germans who were quartered in Ellinikón, a suburb of Athens near the sea, were troubled with sandfly fever. This suburb consisted of substantial houses surrounded by open spaces and gardens. In 1945, the British troops quartered in the same suburb also were considerably affected by the disease. DDT had become available by that time, and its application promptly put an end to the difficulty. In 1948, with no organized spraying for at least a year or two, the writer found that P. papatasii were extremely abundant in some houses.

It was also learned that about one-fourth of the personnel of the United Nations Relief and Rehabilitation Administration living in the Athens area had sandfly fever in 1945. Their quarters were sprayed with DDT in 1946, and no further cases were reported among 2,000 employees. After 1946, the use of DDT had become general in all Greek, American, and British military installations, and from all available reports in 1948, sandfly fever was very rare.

Vector species

In Italy in 1944, P. papatasii was found associated with sandfly fever outbreaks wherever entomologic investigations were made. The species was abundant and was practically the only one found in the affected buildings in Naples and Caserta. A number of buildings in which there had been few or no cases yielded no sandflies. In the Bari section, P. papatasii in small numbers was the only species found. While this species occurs throughout Italy and is

often the dominant species, it is rather irregular in its distribution. For example, in an agricultural village near Naples, sandflies were moderately abundant, chiefly in stables. The principal species was Phlebotomus perniciosus with only a scattering of P. papatasii. These two species were occasionally found in small numbers in buildings or masonry ruins in Rome. In the Fifth U.S. Army area south of the Arno, where there was little sandfly fever among troops, P. papatasii was rare, while P. perniciosus and Phlebotomus perfilievi were fairly abundant, especially in stables. Until actual transmission experiments are undertaken with these and other species, they cannot be ruled out as vectors of sandfly fever. So far as Italy was concerned, however, Army experience confirmed the reported close association of P. papatasii with the disease.

**Strains of virus and immunity**

The Commission on Neurotropic Virus Diseases, Army Epidemiological Board, obtained immunologically identical strains of sandfly fever virus from Sicily and the Middle East in 1943. A strain from Naples obtained in 1944 was found to be distinct, with no cross-immunity with the other strains (p. 167). This has some bearing on the general question of repeated attacks of sandfly fever. Reports from Italy and the Middle East, and information from British sources in regard to West Africa, indicated that second attacks had occurred in the same season, especially following a change of station. In general, the evidence from both civilians and troops who remained in an area for successive years indicated that one attack confers a high degree of immunity.

**Pacific Areas**

No authentic case of sandfly fever is known to have occurred either in Army personnel or in civilians during military operations in the Southwest Pacific, Central Pacific, or Western Pacific Areas. Reports of some cases were made, but none was confirmed on further investigation.

**Japan.**—This was the only area in the Pacific for which intelligence reports indicated that sandfly fever had occurred previously. Reports stated that the disease was “prevalent over practically all of the southern part of the Japanese Empire.” If sandfly fever did occur there, the occupation by U.S. forces was too late in the season to encounter it. Limited inquiry among the responsible medical profession in Tokyo failed to elicit confirmation of the presence of the disease in Japan, though occurrence of dengue and other confusing febrile conditions was admitted.

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21(1) General Report, No. 1 Medical Research Station, Directorate of Biological Research, British Army Medical Services, dated September 1942 to March 1943. (d) Sandfly Fever and Pyrexias of Unknown Origin Resembling It. (2) See footnote 13, p. 117.

22This section on the Pacific areas was written by Lt. Col. (later Col.) Cornelius B. Philip, Surgeon General, Surgeon, Typhus Team, U.S.A. Typhus Commission, Headquarters, Office of the Chief Surgeon, U.S. Army Forces, Western Pacific.

Australia and the Philippines.—In Australia and the Philippines, three and seven species of Phlebotomus, respectively, had been described. Only one species in each country, however, had been recorded as attacking man. The one in Australia is so limited seasonally and geographically as not to constitute a problem in disease transmission. The species in the Philippines has been reported as more common in certain areas at the proper season and was found actually biting troops in the San Jose area of Mindoro at a time when F.U.O.’s were recorded at the 13th and 165th Station Hospitals. Early in the action of the Western Visayan Task Force on the beachhead in this location, 20 cases of sandfly fever for the 4-week period ending on 23 February 1945 were reported to the Office of the Chief Surgeon by a portable surgical hospital. Most of the cases were later proved to be infectious hepatitis in the preicteric phase. Nevertheless, many cases of F.U.O. and dengue-like fever, on which terminal diagnosis could not be changed, continued to be reported as sandfly fever by hospitals in this and other areas of the Philippine Islands. Thirteen iced sera of patients in the febrile stage at the 13th and 165th Station Hospitals, Mindoro, were forwarded by Army courier on 10 April 1945 for study by the Army Epidemiological Board in connection with an experimental assay of sandfly fever and dengue. Results of inoculation of two volunteers with a pool of two of these sera were negative. The occurrence of sandfly fever in the Philippine Islands remains doubtful.

New Guinea.—Many reports of “sandfly bites” were received from troops in beach areas, particularly at Oro Bay and Einschhafen. The only area in the mountains from which similar reports were received was Hollandia. In view of dengue-like fevers and F.U.O.’s continuously reported in troops at the various bases, these reports were closely checked and were found in all instances to be due to minute biting flies related to Culicoides which are often called “sandflies” in the United States. The capability of these flies to transmit sandfly fever is unknown. Two undescribed species of Phlebotomus were found to be abundant in the forested areas over most of New Guinea during and following the rainy season. They were never taken in the act of biting, and troops on maneuvers or patrol in these places never reported bites of a nature attributable to Phlebotomus. It was presumed that the females, which were captured after recent feeding, had fed on reptiles or other local vertebrate fauna and not on man.

Sera from patients with fevers of short duration and doubtful diagnosis were returned from New Guinea by courier for use in volunteers in the aforementioned study. Four strains of typical dengue were recovered, but no serum produced sandfly fever.20

Although cases of sandfly fever were reported at certain bases in New Guinea, there was no presumptive evidence that the disease occurred in troops during action in New Guinea and adjacent islands in the Southwest Pacific Area.

CONTROL AND PROTECTIVE MEASURES

Before the war, no reliable method of controlling *Phlebotomus* was known. In the Mediterranean and the Middle East, the British for many years had practiced certain palliative measures, such as removing rubble, cementing masonry cracks, and treating soil, particularly cracked soil, around barracks with creosote or oil. Protective measures consisted chiefly of the use of fine-mesh bednets, the importance of which has long been recognized. Strong currents of air, either natural or produced mechanically, were found to be of some value. Direct killing by means of sprays was also employed. The only repellent available was citronella oil, of rather limited usefulness.

Repellents

At the beginning of the war, insect repellents became the subject of intensive research by Government agencies and the Armed Forces, chiefly in connection with mosquitoes and malaria. New repellents were adopted by the Army in 1942 and were issued toward the end of that year. Indalone (butopyronoxyl), the first repellent issued, was superseded early in 1943 by the much more effective Rutgers 612 (2-ethyl-1,3-hexandiol), and by dimethyl phthalate. These in turn, as stocks were used up, were replaced by a 6-2-2 mixture of dimethyl phthalate, “612,” and Indalone; this mixture was adopted late in 1943.

Experimental tests.—Studies carried out by the Commission on Neurotropic Virus Diseases, Army Epidemiological Board, in Cairo in 1943 showed dimethyl phthalate to be effective against *P. pupatusii* for a period of 5 to 7 hours. In an experiment involving two groups of soldiers, cases of sandfly fever in a group which used the repellent were markedly fewer than in one which did not.

Tests of repellents “612,” dimethyl phthalate, and the 6-2-2 mixture carried out in Peru in 1944 showed that all three were approximately of equal effectiveness against local species of *Phlebotomus*. They gave protection for at least 3 hours and at times for as long as 5 hours. These repellents, developed for protection against mosquitoes, proved to be even more effective against sandflies.

It was the personal experience of those conducting investigations in Peru, Italy, and Palestine that conscientious use of any of the Army repellents provided complete protection against sandfly bites. However, it was the practically universal experience during the war that it was difficult to get troops to use repellents against either mosquitoes or sandflies except when the men were suffering pronounced annoyance from insect bites.


PROTECTIVE MEASURES IN VARIOUS THEATERS

Prior to the introduction of DDT in 1944, the protective measures against sandflies employed by the U.S. Army consisted of the use of (1) the sandfly net (about 30 meshes to the linear inch) which was issued instead of the mosquito bar made of ordinary netting (about 18 meshes to the inch), (2) repellents, and (3) pyrethrum sprays or the Freon-pyrethrum aerosol bomb. Removing or oiling rubbish heaps and rubble and oiling areas around tents and buildings were occasionally carried out.\(^{33}\)

**India.**—In India in 1943 and 1944, no special protective measures against sandflies were used. Bednets were not of the fine-mesh type. Repellents and insecticides, though used as general measures against insects, were not issued in sufficient quantity until later. In 1945, DDT residual spray was commonly employed throughout the theater, both in military installations and in nearby native dwellings.

**Italy.**—In Italy, antisandfly measures were instituted within a short time after the outbreak of sandfly fever early in July 1944. They consisted chiefly of the use of repellents, sandfly nets, and the aerosol bomb, and, in some cases, spraying with DDT. In the surveys made by the writer in August 1944, it was found that there was considerable carelessness and irregularity in the use, maintenance, and method of using bednets. Also, a number of the nets were of the coarse-mesh type, which provided no protection against sandflies. Repellents were not being systematically applied by the troops. Efforts were made to correct these conditions, with some success. Very few of the U.S. personnel, including medical officers, had any real information about sandflies or even any idea of what they looked like, although in a number of instances sandflies were abundant and in plain sight on smooth white walls near beds. The demonstration to both officers and enlisted men of live sandflies and how to look for them was found to be a great aid in arousing interest and securing cooperation in carrying out protective measures.\(^{34}\)

The results of the Freon-pyrethrum aerosol bomb were not evaluated at the time in terms of sandfly reduction. It is known, however, from studies made in Peru that thorough spraying with pyrethrum or with the aerosol bomb provides a high degree of protection for a number of hours or even an entire night.

**DDT in Italy.**—The residual spraying of quarters with DDT was practiced on an increasing scale in Italy during the summer of 1944, usually in connection with malaria control. By the first of August, it had become virtually routine to use DDT wherever outbreaks of sandfly fever appeared. The DDT, combined with other protective measures, usually caused a prompt improvement of the situation. In studies made during the latter part of the summer,

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\(^{34}\) For the instruction of entomologists, parasitologists, and medical officers, several Italian species of *Phlebotomus* were reared at the 15th Medical General Laboratory. While the technique is not widely known by American entomologists and requires some skill and experience, it has become more or less standard and is described in the literature of experimental studies on the sandflyborne diseases.
it was found that wherever DDT spraying had been adequately carried out, sandfly fever had ceased, and no more sandflies were to be found. The spraying was not always done well. For example, the cavalry barracks at Caserta were sprayed within 2 weeks after the outbreak began, with some improvement in the sandfly fever situation. However, only hand sprayers were available for treating this large structure which had very high ceilings. The result was that only about one-quarter of the necessary quantity of DDT was applied, and the coverage was very uneven. Sandflies continued to be moderately abundant in the building. The spraying was done again, more thoroughly, and the sandflies disappeared.

Controlled experiments were not possible in military installations where all available protective measures were used simultaneously. However, observations at various places in Italy, where DDT spraying was done either in connection with sandfly fever or for other purposes, together with the experimental work to be discussed, warrant the conclusion that DDT residual spray was the most effective single method of sandfly control.

Experimental Studies With DDT

Preliminary experiments with DDT residual spray in Peru in 1941 gave promising results. The flight habits of all known species of Phlebotomus render them extremely vulnerable to the residual action of DDT. They proceed normally by means of very short flights with relatively long pauses, so that in the process of entering a building they spend considerable time on both the outer and inner walls before attempting to feed (fig. 5).

Toxicity of DDT for Phlebotomus.—In Italy, it was found that contact for 2 or 3 minutes with residual DDT caused agitation of sandflies and that in the case of two species a lethal dose was secured within 6 to 15 minutes: P. papatasii, however, required from 15 to 30 minutes.

Experiments, Naples. Spraying the outer walls of stone stables in a village near Naples reduced to stragglers the sandflies which could be found inside, while normal numbers were found in untreated buildings. P. perniciosus was the principal species in this area. The effect lasted throughout the 4 to 5 weeks of observation.

Experiments, Palestine.—In Palestine, controlled experiments were carried out near the Dead Sea in October and November 1944 where P. papatasii is extremely abundant (fig. 6). It was found that spraying the inner walls and ceilings of buildings provided complete control, with no bites reported, while spraying the outer walls or merely the doors and windows, together with a foot or two of the wall surrounding such openings, reduced sandflies by about 75 percent and gave comparative freedom from bites. An experiment with sprayed tents, although interrupted, indicated that the results would be comparable. It was recommended that rooms be sprayed inside, together with open-
ings and a little of the surrounding outer wall (fig. 5). In northern Palestine, a stone wall from which numerous sandflies (*Phlebotomus major*) emerged at night yielded no sandflies after spraying, while normal numbers were caught on an unsprayed wall nearby. This experiment indicated that DDT could deny to sandflies their customary outdoor resting and breeding places.

**Area control with DDT**

**Peru.**—Experimental studies carried out in Peru in 1945 indicated that area control (that is, control of sandflies outdoors as well as indoors) could be obtained within a radius of 100 to 200 meters by spraying with DDT those outdoor structures, especially loose-laid stone walls, which harbor sandflies or serve as breeding places, or on which sandflies alight in flying toward a blood meal (fig. 7). Such surfaces, if properly selected, would not only eliminate the principal resting and breeding places but would form a series of lethal barriers to the movement of sandflies within the area (figs. 4, 6, and 7).

In one experiment, the combined spraying of a house and surrounding walls virtually eliminated sandflies on the premises, an effect which persisted for over a year and a half, while in houses 75 to 200 meters distant sandflies
were normally abundant. As part of the Peruvian work in 1945, practical control projects were started in two large construction camps and then carried on by the engineers in charge. Residual DDT was applied to the interiors of buildings and also to some outdoor walls. The sandflyborne diseases were bartonellosis and some cutaneous leishmaniasis. Both ceased to be problems. On visits to Peru by the writer in 1947 and 1950, it was learned that control of both sandflies and disease continued to be complete. One project had terminated in 1946, but another had been started in 1947. Two projects were in operation in 1950. There had been no bartonellosis or leishmaniasis among the 3,000 workmen exposed.

**Greece, Crete, Sardinia, and Italy.**—The writer has had other opportunities of confirming the effectiveness of residual DDT in controlling *Phlebotomus*. Observations in Greece, both on the mainland and in Crete in 1948 (p. 122), showed that the application of residual DDT on the inner walls and ceilings of houses (primarily for malaria control) had reduced *Phlebotomus* within the sprayed villages almost completely, while they were abundant in some unsprayed places. Most of the villages had been sprayed during 3 consecutive years. It was the universal testimony that sandflies had been abundant but had "disappeared" with the first spraying. The dominant sandfly in unsprayed areas was *P. papatasii*. During the same summer, it was found both in Sardinia and in the Italian Province of Abruzzi that the application of DDT had had a similar devastating effect on *Phlebotomus*. 
Sandfly control: General recommendations

From these various experiments and observations with several species of *Phlebotomus* in both tropical and temperate regions, the writer considers it established that sandflies are extremely vulnerable to residual DDT and that a single annual application, preferably before the sandfly season, will give virtually complete control within dwellings or other buildings and, in the case of compact communities, within the area as well.

For military operations within a sandfly area, the writer would recommend that in addition to the conscientious use of the protective measures already discussed—repellents, sandfly net, aerosol bomb—the interiors of all structures or tentage, but especially of living quarters and animal shelters, be treated with residual DDT before the sandfly season, or as soon as possible if the season has already begun. The danger from sandfly fever would be especially great in buildings recently vacated or still occupied by local people, since such places would have the greatest concentration of both sandflies and sources of infection.

Jungle or forest.—In jungles or forests, the data are rather scanty in regard to sandfly control. However, with the exception of leishmaniasis in South and Central America, there are not known to be any serious sandfly problems in such habitats. The spraying of jungles from the air, which was extensively investigated and practiced for mosquito control during World
War II, would also doubtless destroy many adult sandflies along with the mosquitoes. This was indicated by incidental observations made by the writer on sandflies during airspray experiments carried out by the U.S. Army in Panama in 1945, but the data are not conclusive since the experimental areas to begin with had relatively few sandflies.

In the event of military operations in a forest area where sandflies were disease transmitters or pests, residual DDT on tentage or structures could be expected to give indoor protection. In addition, the writer considers it probable that considerable reduction of sandflies in the immediate area could be achieved by spraying exposed rocky surfaces and the buttresses and trunks of larger trees, with particular attention to hollow trees and to places where animals were picketed or corralled. This statement is based on the writer’s observation on the habits of sandflies in forests both in Panama and in Paraguay. They commonly alight on larger objects in their path, which could be rendered lethal by DDT.

Airspray.—The effect of airspray in village or urban areas may merit investigation. A suburb of Athens was sprayed from the air during the summer of 1946. There was consistent local testimony that sandflies had been abundant but ceased to give annoyance. Since a method of this type might have military applications, it would be desirable to determine experimentally the limits of its usefulness.

**SUMMARY**

Of the sandflyborne diseases, only sandfly fever was an important military problem during World War II. A general account of the epidemiology and military history of the disease is given. Over 18,000 cases were reported in U.S. troops from the Middle East, Asiatic, and Mediterranean theaters (table 20). Undoubtedly, a great many additional cases were reported as F.U.O.’s, and it is probable that the total of sandfly fever cases approached 24,000. Areas with particularly high rates were Sicily (during the invasion in 1943), and the Persian Gulf Command.

The greatest incidence among troops in actual combat was during the Sicily Campaign in the summer of 1943. Military operations were also hampered by sandfly fever in Italy in 1944.

Sandfly fever occurred only in known endemic areas, with the exception of the eastern part of India and parts of Burma where it had not previously been recognized. Association with *P. papatasii*, known to be a vector, was demonstrated in Italy and the Middle East. Species of *Phlebotomus* were present in all areas where the disease occurred.

Special studies on the etiology and transmission of sandfly fever were carried out in the Mediterranean and Middle East theaters in 1943. Two immunologically distinct strains were isolated. Epidemiologic and entomologic studies were made in Italy and Palestine during 1944.

In all theaters, the standard protective measures against biting insects, including mosquitoes and sandflies, were available. These consisted chiefly
of repellents, sandfly nets, and the Freon pyrethrum aerosol dispensers. These materials, when properly used, were proved to be effective, but their application was sometimes irregular. Control studies made in Italy, Palestine, Peru, and Greece showed that the DDT residual spray was extremely effective against sandflies. The method of applying residual DDT, which has become standard in malaria control, namely, spraying the inner walls and ceilings of dwellings and animal shelters, also gives virtually complete protection against sandflies indoors and, in the case of compact communities, outdoors as well as within the treated area.

**Part II. Experimental Studies**

*Albert B. Sabin, M.D.*

**HISTORICAL NOTE**

All that was known with certainty in 1940 concerning the virus of sandfly fever was that it was a filterable agent present in the blood of patients 1 to 2 days before and for 1 day after onset of the fever, and that *P. papatasii* was capable of transmitting the infection from man to man. Since the clinical manifestations of sandfly fever are not in themselves sufficiently characteristic to permit identification of an unknown, filterable agent, it was clear to the critical investigator that primary identification of the virus of sandfly fever was a difficult matter which could not be regarded as complete without the demonstration of its transmissibility by *P. papatasii*. Since this type of critical identification was apparently too difficult for most investigators, the literature contained a good many incomplete observations and conclusions based on inadequate data which at best left the subject in a somewhat confused state.

The work reported by Shortt and his associates* in India between 1934 and 1939 was particularly intriguing, although inconclusive. These investigators reported that human infectious serum (presumably containing the sandfly fever virus) produced a febrile illness in *Macaca rhesus* monkeys. Although the blood of such monkeys upon inoculation in human volunteers produced, after an incubation period of 5 to 7 days, only vague symptoms of headache and generalized malaise without distinct fever (which might have been mild serum sickness), and although no studies on the leukocytes were carried out nor passage to other volunteers attempted, it was, nevertheless, concluded that sandfly fever "appears" to have been transmitted to monkeys.

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SANDFLY FEVER

Shortt and various associates also reported that infectious human serum (again presumably containing the sandfly fever virus) produced lesions on the chorio-allantoic membrane of embryonated eggs, which could be transmitted in series with filtered extracts of these membranes and be prevented from developing by the serum of patients convalescent from sandfly fever. They concluded from these data that "the virus of sandfly fever has been cultivated by the chorio-allantoic membrane technique and in tissue culture and the cultures carried through numerous passages." Then, on the assumption that the production of lesions on the chorio-allantoic membrane could be used as an indicator of the presence of virus, these investigators reported on various properties of this virus. Thus, the virus was said to have a size of 160 mg. 27

In a communication presented at the Third International Congress of Microbiology in 1939, Shortt and his coworkers 28 summarized their studies on the sandfly fever virus as follows:

This virus has been maintained in culture on the chorio-allantoic membrane of chick embryos and in tissue culture for 61 and 41 passages, respectively.

The cultured virus has been shown to possess no pathogenic properties for man and laboratory animals, whatever the route of inoculation.

The virus has been demonstrated in the bloodstream of monkeys inoculated subcutaneously up to a maximum of 19 days. In human cases of the disease occurring naturally, the virus is usually demonstrable in the bloodstream for 7 days, and in one case was demonstrable for 28 days.

In the case of inoculated monkeys, neutralizing antibodies have been found present up to at least 69 days. Attempts at the prophylactic inoculation of human beings with a vaccine containing live virus have been made. Two doses of vaccine, with a week's interval between doses, were given. In the inoculated persons, the presence of virus circulating in the peripheral blood was demonstrated 5 days after the second dose of vaccine. Thirty-five days after the second dose of vaccine the sera of some of the vaccinated persons showed the presence of neutralizing antibodies, while those of others similarly vaccinated failed to do so. Infection of the vaccinated persons and controls with infective serum from sandfly fever cases gave inconclusive results.

Thus, although the cultured virus was not pathogenic for man and the infection experiments in persons vaccinated with this cultured material gave inconclusive results, these investigators did not entertain the conclusion that they might not be dealing with the virus of sandfly fever. Furthermore, although in experiments on human volunteers the virus of sandfly fever had not been found beyond 24 hours after onset of the fever, the chorio-allantoic membrane technique seemed to reveal the presence of a lesion-producing agent (virus?) for as long as 7 days and, in one instance, for 28 days.

While the experiments of Shortt and his coworkers with chick embryos were thus inconclusive, they were, nevertheless, followed by reports of Russian workers in 1940 and 1941 which, however, did not become available to us until

27 The figure of 160 mg should actually have been given as 190 to 285 mg, since the membrane with an average pore diameter of 450 mg just permitted the "activity" to pass, while the 380 mg membrane held it back. Using Elford's generally accepted formula, the particle size should have been taken as \( \frac{1}{2} \) to \( \frac{3}{4} \) of 380 mg.

they were abstracted in the *Tropical Diseases Bulletin* in April 1943. In the 1940 report, Demina and Levitanskaja stated not only that the sandfly fever virus (serum of patients with the disease) produced lesions in the chorio-allantoic membrane, which could be prevented by immune serum, but also that the whole membrane and whole embryos produced “typical phlebotomus fever” in eight human volunteers. It was added, however, that “injection into seven volunteers of [suspensions of] pieces of chorio-allantoic membrane taken near the site of inoculation (from primary and subcultures) failed to produce infection or immunity.” In the 1941 report, Demina stated that sandfly fever virus inoculated directly into the yolk sac of chick embryos produced cultures in which both the chorio-allantoic membrane and embryo were virulent, as demonstrated by successful infection of mental patients. The abstract further went on to say:

** Of the two strains maintained by her, one was virulent after 30 subcultures, the other after 20, though in both the virulence was continuously manifested only till the tenth subculture. Later passages behaved in an irregular manner, some losing not only their virulence but also their antigenic properties.

Aside from these rather conflicting reports on the behavior of the sandfly fever virus in chick embryos, the available data indicated that no clinically apparent disease was produced in guinea pigs, rabbits, or dogs as a result of extraneural injection of human serum containing the virus. There was no record that the method of intracerebral inoculation of rodents had been explored.

As regards the natural history of the virus, nothing was known of its possible presence in hosts other than man and *P. papatasii*. It was assumed that the virus persisted in nature as a result of transovarian passage from one generation of infected *Phlebotomus* to another—an assumption which received considerable support from the rather detailed experiments on human volunteers reported in 1937 by the Russian investigators, Moshkovsky and his associates.

### OBJECTIVES OF RESEARCH

The high incidence of sandfly fever among British troops stationed in Palestine and the Middle East since 1939 and its appearance among American troops in the Middle East and the Persian Gulf Commands, as well as in the Asiatic theaters in 1942 (p. 118), led the Commission on Neurotropic

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43 See footnote 15, p. 118.
Virus Diseases, Army Epidemiological Board, to undertake experimental studies on this disease. The first group to concern itself with this work consisted of Dr. John R. Paul, Director of the Commission; Maj. (later Col.) Cornelius B. Philip, SnC, an entomologist; and this author, a virologist. This group of workers made a preliminary survey of the disease in the Middle East and Palestine and set up an experimental ward and laboratory as part of the 38th General Hospital at Camp Russell B. Huckstep in the desert approximately 8 miles outside of Cairo. One building was especially altered for this purpose and screened with special care against sandflies. It was ready for use on 20 May 1943 and was maintained as an active laboratory until 15 December 1943. The objectives of research on this disease were as follows:

1. As an immediate step, to determine whether any of the available mosquito repellents might be effective in protecting against the bites of P. papatasii, a procedure which might then be used in an attempt to protect against this disease.

2. To recover one or more strains of the virus of sandfly fever by reproduction of the disease in human volunteers and to make positive identification by transmission through P. papatasii raised in the laboratory from ova of flies previously proved to be noninfective.

3. To attempt to infect a large variety of lower animals and to cultivate the virus in embryonated eggs, simultaneously with the work on infection of human volunteers.

4. To develop an adequate source of the virus from lower animals, embryonated eggs, insects, or human beings, which might be used for the elaboration of a specific diagnostic test as well as for studies on artificial immunization against the disease.

5. To investigate the possibility of transmission by vectors other than P. papatasii, especially parasitic arthropods indigenous to epidemic zones and mosquitoes prevalent in the United States.

EFFECTIVENESS OF REPELLENTS UNDER NATURAL CONDITIONS

In May 1943, nothing was as yet known regarding the effectiveness of DDT against P. papatasii. It was also clear at that time that no matter how successful the experimental studies on sandfly fever during the coming summer might prove to be, they would have nothing to offer in the way of specific biologic control during the 1943 season. An immediate investigation of the possible value of insect repellents then available to the Army in large quantities was, therefore, indicated as a measure which might be of practical value in the control of the disease in the forthcoming months. This study was carried out with the help of Prof. S. Adler and Mr. S. Arkin, of the Hebrew University in Jerusalem. Lt. Col. C. H. S. Little, British Deputy Director of Medical Services in Palestine, not only lent his cooperation but also served as a volunteer in the studies. Two repellents, greaseless and practically odorless after application, were investigated: (1) A British preparation consisting
of a vanishing cream containing pyrethrum, and (2) an American fluid preparation (called Skat commercially) containing dimethyl phthalate. Professor Adler pointed out, and it was later confirmed by observation, that during a period of study as short as 15 minutes the largest number of bites and feeds might be expected from *Phlebotomus* contained in a closed test tube, a lesser number from flies in an open gauze-covered tube, and least of all from flies in a fairly large muslin-covered wire cage. Areas of skin (about 1.5 cm. in diameter; that is, the internal diameter of the test tube) on the flexor surface of the forearms either treated with repellent or untreated were to be exposed to approximately 20 unfed *Phlebotomus*. While this method of testing did not reproduce natural conditions, it, nevertheless, provided a technique by which the effectiveness of different repellents could be estimated and compared. To supplement these tests, the same repellents were also studied under natural conditions in sleeping rooms occupied simultaneously by treated and untreated individuals. The large numbers of *Phlebotomus* flies required for these tests could be obtained on short notice at that time of the year only at the Dead Sea post where quarters and facilities for this work were obtained through the courtesy of the Palestine Potash Company. Mr. Belfer, the malaria control officer of the company, not only made all the arrangements for the working and sleeping rooms but also helped find and catch the *Phlebotomus* which were needed for the experiments. Seven American volunteers of the Levant Service Command, attached to the 24th Station Hospital in Tel-Litwinsky, Palestine, participated in this study.

The closed-tube tests summarized in table 21 indicated that both the British and American preparations possessed repellent properties but that the effects produced by them under the special experimental conditions were somewhat different:

1. The protection afforded by dimethyl phthalate, while it lasted, was almost complete against both biting and feeding. With the British cream, however, the bites were greatly reduced in number but not entirely prevented although, with few exceptions, it so affected the treated skin that the flies failed to feed on it for a longer period than in the case of the dimethyl phthalate.

2. With dimethyl phthalate, the period of protection ended, that is, the flies bit and fed again as on the control areas, at 6 1/4, 7, and 8 hours, respectively, in the three subjects. With the British cream, a definite endpoint was not established because the protection was only partial against biting throughout the period of the test, but still practically complete at the end of 9 hours as regards the failure of the flies to engorge on the treated skin.

The open-tube tests were unsatisfactory except in two volunteers who were treated with dimethyl phthalate. The results again indicated that this preparation protects for 6 to 7 hours but not for 8 hours under the conditions of these tests. The impression gained from the control tests under natural conditions in sleeping quarters was that both the dimethyl phthalate and the British cream, when properly applied to all the exposed skin surfaces, may
Table 21.—Repellent tests against P. papatasii, closed-tube method

<table>
<thead>
<tr>
<th>Repellent tested</th>
<th>Hours after application</th>
<th>Effect of applying approximately 20 female flies first to treated and then to untreated site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Subject A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>Dimethyl phthalate........</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>British pyrethrum cream...</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

Notes: The italic figures represent the behavior of the flies on the untreated skin and are the control for evaluating the effectiveness of the repellents tested.

give adequate protection against the bites of P. papatasii during the usual 8-hour period of sleep (table 22).

None of the exposed American volunteers developed sandfly fever, suggesting that the Phlebotomus which were used either had a very low infection rate or were uninfected—a factor of importance since the progeny of these flies were subsequently used for the experimental transmission tests in the desert laboratory.

As a result of these studies and in the absence of any specific immunologic methods of control, it was believed that for 1943 repellents might probably be used with profit in attempts to reduce noneffectiveness due to disease and loss of sleep resulting from the bites of P. papatasii. The studies indicated that in routine practice two applications of the repellent would be required, one at sunset and one before retiring. It was estimated that the total amount of liquid repellent required per man per day would be about 10 cc.

44 See footnote 7, p. 110.
Table 22. Effect of repellents under natural conditions in sleeping quarters at night

<table>
<thead>
<tr>
<th>Room</th>
<th>Subject</th>
<th>14 May 1943</th>
<th>15 May 1943</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td>Result</td>
</tr>
<tr>
<td>1</td>
<td>S</td>
<td>Untreated</td>
<td>Unable to sleep because of innumerable bites all through the night until 6 a.m. when subject arose.</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>British cream applied 12:30.</td>
<td>Slept through night; 1 bite on arm, 7 a.m., 1 bite on ankle, 8 a.m.</td>
</tr>
<tr>
<td></td>
<td>Sa</td>
<td>Untreated</td>
<td>Unable to sleep because of innumerable bites.</td>
</tr>
<tr>
<td>2</td>
<td>Se</td>
<td>British cream applied 10:45 p.m.</td>
<td>No bites felt; slept right through night.</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>Dimethyl phthalate, 11 p.m.</td>
<td>No bites; slept right through night.</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>Untreated</td>
<td>Felt no bites; slept well.</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>do</td>
<td>do.</td>
</tr>
<tr>
<td></td>
<td>Do</td>
<td>British cream</td>
<td>do.</td>
</tr>
<tr>
<td></td>
<td>Dr</td>
<td>Dimethyl phthalate.</td>
<td>do.</td>
</tr>
</tbody>
</table>

1 It was a cool night and subjects completely covered themselves with blankets.

Note.—Approximately 150 uninfected female P. papatasii liberated in each room on the night of 14 May. On the night of 15 May, approximately 140 were liberated in room 1, 135 in room 2, and 200-250 in room 3.

The effectiveness of dimethyl phthalate for the control of sandfly fever under natural conditions was tested during a small outbreak among Americans quartered in an inhabited area of Cairo, Egypt, during September and October 1943. The results, published in detail by Philip, Paul, and Sabin were as follows:

The men in a given barracks were divided into two groups. The repellent was issued to one group (82 men to begin with) and an inert control solution to the other (88 men to
begin with). Directions for the application of these solutions when the men retired at night were the same for each group. Of the men receiving the repellent, 42 percent reported relief from bites; of those receiving the control solution, 12 percent reported relief. Of the men receiving the repellent, 2 acquired sandfly fever during the 5-week experimental period; and of those receiving the control solutions and no solution, 12 acquired sandfly fever. Both men who "used" the repellent and acquired sandfly fever acknowledged that they had not followed directions and had failed to apply it for several consecutive nights prior to acquisition of the disease. * * * Dimethyl phthalate as an insect repellent is recommended as a method for the control of sandfly fever. It has been tested during an epidemic and shown to be of apparent value.

STUDIES ON AMERICAN VOLUNTEERS IN EGYPT, 1943

Recovery of agent.—The source of sandfly fever virus was the blood of patients in the first 24 hours of the disease. The patients came from among British troops who had contracted the disease in the vicinity of Cairo and from among U.S. troops stationed at Deversoir Field on the Suez Canal. Approximately 50 cc. of blood was obtained from each patient; the serum was separated and stored in the frozen state at the low temperature produced by solid CO2. The following points were established in each case before any serum was included in the pool that was to be inoculated into the human volunteers:

1. The subsequent clinical source of the disease had to be compatible with that of sandfly fever.
2. The presence of *P. papatasi* in the area from which the patient came had to be demonstrated by a member of the Virus Commission.
3. The patient's past history should have had nothing to contraindicate the use of his serum in other human beings—syphilis and jaundice having been especially ruled out.
4. A negative Kahn test and bacteriologic sterility had to be established.

When a sufficient amount of serum satisfying these criteria had been accumulated, a pool derived from 11 patients was prepared, and on a selected day the pooled serum was inoculated into human volunteers, monkeys, hams ters, white mice, gray mice, desert rats, rabbits, and guinea pigs. The same pooled serum was also inoculated by different routes into embryonated eggs onto the chorio-allantoic membrane, into the allantoic sac, or into the yolk sac.

The human volunteers were chosen from among U.S. troops without a previous history of sandfly fever who had arrived from the United States after the last sandfly season. They were quarantined in special, air-conditioned, sandfly-proofed rooms for a period of 10 days, the maximum known incubation period of the disease. At the end of this period of quarantine, during which baseline observations were made on temperature, pulse, and leukocyte count, each of the first four volunteers was inoculated with 1 cc. of the pool of acute sandfly fever serum—0.1 cc. intracutaneously and 0.9 cc. subcutaneously. After an incubation period of 4 to 6 days, three of the four volunteers developed the typical symptoms and fever of the natural disease (chart 3). During the course of subsequent experiments with insects, five additional volunteers were
CHART 3.—Experimental sandfly fever in American volunteers in Egypt
each inoculated with 0.3 cc. of the same pool of acute sandfly fever serum, and all developed the typical experimental disease after the usual incubation periods. As each volunteer developed this fever, 50 to 75 cc. of blood was obtained, and the frozen serum was saved as a supply of potentially infective virus. During the course of certain other experiments, two human volunteers were inoculated with a pool of serum from the first group of volunteers and both developed typical experimental sandfly fever after the usual incubation period. Thus, it was possible to demonstrate not only that an infective agent was obtained from the serum of patients with the naturally occurring disease but also that the bacteria-free agent was capable of transmission in series. While the circumstantial epidemiologic evidence taken together with the clinical manifestations of the experimental disease strongly suggested that the agent recovered was the virus of sandfly fever, it was, nevertheless, still necessary to satisfy another criterion; namely, that of transmission by P. papatasii.

**Hepatitis virus in pool of serum.**—It should be noted here that despite all the care that was taken in the selection of the original group of patients with the naturally occurring disease, the original pool of serum that was used to inoculate the first human volunteers contained, in addition to the infectious agent of sandfly fever, another agent capable of producing hepatitis with jaundice. Of the 10 human volunteers who were inoculated with this pool for the transmission of sandfly fever, 4 also developed hepatitis with jaundice 72 to 94 days after the first inoculation. The same pool of serum was subsequently injected parenterally in human volunteers in the United States and three of the five developed hepatitis with jaundice. A further complication occurred when the serum of one of these volunteers in Egypt, obtained 34 days after inoculation with the original pool and 60 days prior to his development of hepatitis with jaundice, was used after heating at 56° C. for a half hour as control skin-test material in eight of the personnel associated with the hospital and laboratory of the Commission. Three of these eight individuals developed hepatitis with jaundice after incubation periods of 94 to 132 days. The details of this intercurrent hepatitis experience were reported by Paul, Havens, Sabin, and Philip.46

**Experimental transmission of sandfly fever agent by P. papatasii.**—The P. papatasii used for these tests were reared in the Commission laboratory from ova derived from the stock collected in May 1943, in the Dead Sea area during the experiments on insect repellents. Because of the restricted residence of the local population and because the parent flies failed to produce the disease in eight American volunteers, who were bitten by large numbers of them, the stock of sandflies was regarded as uninfected and, therefore, especially suited for the transmission experiments. The other bloodsucking insects used in the first test consisted of fleas (Pulex irritans) collected from native clothing in an Egyptian village, and Culex pipiens mosquitoes collected at Deversoir.

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Field during an outbreak of sandfly fever among American troops. These three species of bloodsucking insects were fed on three of the first group of human volunteers during the first 24 hours of the experimental disease. After extrinsic incubation periods which varied from 8 to 18 days, the survivors were allowed to bite human volunteers—two subjects being used for each species of insect. One volunteer, on whom 13 *P. papatasii* had fed developed typical sandfly fever after an incubation period of about 3 1/2 days, while another, on whom 21 engorged one or more times, exhibited no signs of the disease. None of those bitten by either *P. irritans* or *C. pipiens* developed the disease. In the *P. irritans* tests, 23 insects fed one or more times on each of the two volunteers, while in the case of *C. pipiens*, 11 insects fed one or more times on one volunteer and 7 insects one or more times on the other. It should be noted that the five volunteers who failed to develop the disease were subsequently shown to be susceptible when they developed typical sandfly fever after an intracutaneous injection of 0.3 cc. of the infectious serum constituting the pool obtained from the original lot of naturally infected patients.

In a subsequent experiment, another lot of sandflies reared from ova in the laboratory was fed on two volunteers who were inoculated with the serum obtained from individuals with the experimentally induced illness. After suitable incubation periods, these sandflies were allowed to feed on two additional human volunteers, one of whom developed typical sandfly fever (bitten by 13 flies), while the other (bitten by 12 flies) failed to develop the disease. Thus, in two separate experiments, it was possible to demonstrate that the infectious agent which was capable of reproducing the manifestations of naturally occurring sandfly fever was also transmissible by *P. papatasii*, but not by *C. pipiens*, *P. irritans*, and in later experiments also not by *Aedes aegypti* (chart 4). It is noteworthy that other sandflies reared in the laboratory, which were not allowed to feed on infected patients, were found to be free of the virus, as demonstrated by tests on two human volunteers who were bitten by 56 and 91 flies, respectively. At least one of these two volunteers was later shown to be susceptible to the virus when he developed the disease following a parenteral inoculation of infectious serum. It should be noted here that additional experiments with *C. pipiens* also yielded negative results in three volunteers. The extrinsic incubation periods were purposely prolonged (1) to compare with the experience with yellow fever in which unfavorable mosquito hosts may become infectious after a longer extrinsic incubation period than is required for *A. aegypti*, and (2) in case occult virus was present which needed stimulation by repeated blood meals. The conclusion from these tests was that, excepting *P. papatasii*, none of the other bloodsucking insects which were tested could play important, if any, roles as vectors of this virus.

The question of interstadiol or transovarial transmission of the virus was tested in the Commission laboratory, as follows: (1) *P. papatasii* larvae were allowed to ingest lyophilized virus (human serum) and the resulting adults were tested on human volunteers with negative results, and (2) *P. papatasii* hatched out in the laboratory from ova derived from parent females of proved
infectious capacity as late as 8 to 10 days after the infectious blood meal failed to produce the disease in human volunteers. While these experiments were not extensive, they indicated that the virus did not pass from generation to generation in all infected flies; however, it was realized that this question could not be regarded as having been settled, particularly in view of Whittingham's 17

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and Moshkovsky's experiments which still left the possibility that such an event might occur occasionally.

**Attempted propagation of the virus.**—Using human serum, whose pathogenicity was established by simultaneous tests in human volunteers, inoculations were performed by the intracerebral, intranasal, intracutaneous, subcutaneous, intratesticular, and intraperitoneal routes in monkeys, young hamsters, young albino mice, wild gray mice, desert rats, rabbits, and guinea pigs. In the first series of tests, there was no suggestion of reaction in any of the animals except the hamsters, where it is probable that some infectious agent was carried for two passages before it was lost.

Two of the six hamsters which were first inoculated succumbed with nervous signs on the fifth day. One died and the other was sacrificed, and suspensions of their brains and viscera, which were bacteriologically sterile, were passaged into three new hamsters. One of these three again succumbed with similar severe nervous signs 3 days after inoculation. Although its brain and viscera were bacteriologically sterile, there was histologic evidence of acute ependymitis and meningitis as well as focal necrotic and inflammatory foci in the liver. Nevertheless, further passage into new hamsters was negative, as were also repeated inoculations of the material from the first two hamsters into six new ones, and of the original pool of acute sandfly fever serum into six additional hamsters. In addition to these tests, hamsters were also inoculated with whole blood or serum from other acute cases of natural or experimental sandfly fever as well as with a suspension of infected *P. papatasii*, but all with negative results. The total number of hamsters used in these tests was 58, and all that one can conclude is that no clinically apparent infection can be produced with regularity in these animals by the sandfly fever virus. Since no tests on human volunteers were carried out, one can say nothing about the possibility of clinically inapparent propagation of the virus in this species.

The susceptibility of white mice was also tested extensively in 71 animals, using the same material inoculated in hamsters. The results were all negative including blind passage with the brains and lungs of the inoculated mice.

Ten monkeys representing five different species were inoculated intracerebrally, intracutaneously, subcutaneously, and intraperitoneally with infectious serum or whole blood from cases of natural or experimental sandfly fever. There were three grives (*Cercopithecus griseoceividis*), two vervets (*Cercopithecus aethiops centralis*), two red African hussars (*Cercopithecus erythrocebus* patas), one *Macaca radiata* from India, and two young baboons (*Papio hamadryas*). No fever or other clinical manifestations of disease were observed in any of them. With the exception of the three grives, which had been in Cairo for at least 1 year, and the *M. radiata*, whose history was unknown, the other monkeys were brought by plane from regions of Africa which are presumably free of sandfly fever and *P. papatasii*.

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*See footnote 44, p. 134.*
In view of the reports and conclusions of Shortt and others that the virus of sandfly fever had been cultivated on the chorio-allantoic membrane of embryonated eggs with the production of plaques which could be prevented by convalescent serum, special efforts were made with this method. Among twenty-two 10-day embryonated eggs inoculated in this manner with the pool of acute sandfly serum of proved potency and with the sera from five acute cases of the natural or experimental disease, all embryos survived and only one egg exhibited a single plaque on the chorio-allantoic membrane. On passage of a suspension of this membrane into four new eggs, one egg again showed an opaque zone on the chorio-allantoic membrane, but the same was found in one of three control eggs which were inoculated with the 5 percent normal chicken serum-Tyrode’s solution which was used to suspend the original membrane.

Five blind passages at 4-day intervals were carried out in eighteen 8-day eggs inoculated into the allantoic sac and six passages at similar intervals in twenty-three 6- to 7-day eggs inoculated into the yolk sac. No evidence of pathogenicity for the chick embryo was observed. Inoculation of hamsters and white mice with the first and fifth passage allantoic fluid and fourth passage yolk sac yielded negative results. Negative results were also obtained when the allantoic fluid and yolk sac suspensions were used as antigens in complement fixation tests with convalescent sera from patients with the experimental disease. Thus, although volunteers were not inoculated with any of the chick embryo material, making it impossible to conclude that the virus did not multiply in the embryonated eggs, it was, nevertheless, apparent that the chick embryo could not be used either for identification of the virus or for detection of the specific antibody, as the reports of Shortt and his associates had led one to hope.

Search for a specific or nonspecific diagnostic test.—Precipitin, complement fixation, and hemagglutination tests were tried using the acute stage serum obtained from natural or experimental cases of the disease, or an extract of P. papatasii fed on experimentally infected volunteers as the antigen, and convalescent sera from natural and experimental cases as the antibody. All yielded negative results. An attempt was made to determine whether or not a skin test might be devised by testing the effect of fresh and heat-inactivated infections sera in normal individuals and in volunteers convalescent from the experimental disease. However, no significant skin reactions were obtained in any of them. Nonspecific tests in the form of sheep cell agglutinins and cold agglutinins were also investigated with negative results.

Preliminary observations on immunity to homologous virus.—Two preliminary tests were carried out on the volunteers in the desert laboratory. In the experiments with various vectors, the volunteer who developed the experimental disease after being bitten by infected P. papatasii was found to be immune when tested with infectious serum 17 days after the onset of his fever, while five other volunteers, inoculated simultaneously with the same dose, all developed typical sandfly fever. The volunteers used in the very first
experiment on infectivity of the original pool of sandfly fever serum were each
reinoculated with 0.1 cc. of the same infectious serum 14 days after the first
inoculation, or 8 to 10 days after the first day of fever, and only one exhibited
a rise in temperature of 1° F. 3 to 4 days after inoculation unassociated with
any symptoms. However, since there were no simultaneous controls for this
test, the negative results could not be regarded as conclusive, and it was evident
that the question of immunity still remained to be investigated experimentally.

**Summary of work accomplished and problems requiring further study.—** Perhaps the most important achievement in this laboratory was the
accumulation of a considerable amount of infectious serum in which the exis-
tence of the virus of sandfly fever was established not only by serial trans-
mision experiments but also by the proof of its transmission by *P. papatasii.*
The hope that some simple laboratory animal or embryonated eggs could
serve as indicators of the presence of this virus was unfortunately dispelled
by the preliminary experiments that were carried out. In addition to further
attempts at adaptation of the virus in experimental animals and embryonated
eggs, it was believed of the greatest importance to learn more about immunity
to this virus under experimental conditions. In view of the conflicting reports
concerning the immunity which follows an attack of the natural disease, it
was deemed advisable to learn what immunity may result from an experi-
mental attack of the disease when the same strain was used for challenge in
individuals living in sandfly-free regions. It was also desirable to determine
whether or not doses of the virus, too small to produce the disease, would
prove to be immunogenic and whether or not inactive virus could produce
immunity. It also seemed of interest to determine whether or not serial
passage of the virus in human beings without intervention of the natural
insect vector might perchance cause sufficient attenuation to permit its use
for immunogenic purposes. Twenty-seven American enlisted men served as
volunteers in the studies carried out in the desert laboratory.

**STUDIES ON HUMAN BEINGS IN THE UNITED STATES**

Since *P. papatasii* is not present in the United States and since its im-
portation was prohibited, it is clear why the initial, orienting experiments
just reported were best carried out in the desert laboratory in Egypt. It
was also clear, however, that the more extensive work involving the use of
larger numbers of human subjects as well as the definitive studies on im-
munity could be carried out better in the United States where American
civilians might become available as volunteers and where the studies on im-
munity would not be complicated by the question of spontaneous reinfection,
perhaps inapparent infection, in nature. Accordingly, through the cooper-
ation of the authorities of Longview State Hospital in Cincinnati, Ohio, and
with the special cooperation of its medical director, Dr. Douglas Goldman,
the patients in that hospital requiring some form of fever therapy, with the
consent of their families or guardians, became available for tests on materials
suspected of containing virus of sandfly fever. The work was carried out by
the author in his laboratories at the Children's Hospital Research Foundation
and the Department of Pediatrics of the University of Cincinnati College of
Medicine, Cincinnati, Ohio. In addition to the patients who were available
at Longview State Hospital between October 1943 and April 1944 and again
between June 1945 and the end of 1945, a number of tests were carried out
on human volunteers among the prisoners of the New Jersey State Prison
at Trenton, N.J., between May 1944 and May 1945.

Isolation of Sicilian Strain of Virus and Its Identification by Cross-Immunity
Tests With Middle East Strain

A field investigation which this author carried out during the Sicily
Campaign (pp. 168–174) indicated that an estimate of approximately 8,500 cases
of sandfly fever was reasonable for the period of 10 July to 3 September 1943,
among the personnel of the Seventh U.S. Army. 8 It seemed highly desirable,
therefore, to establish by laboratory methods the nature of the etiologic agent
responsible for the febrile illness that was seen more often than any other
disease during that period in Sicily. Accordingly, blood was obtained within
24 hours after onset from three patients who, at the time, were in the 91st
Evacuation Hospital in Palermo, Sicily. The serum was at the prevailing
room and outdoor temperatures for a period of about 40 hours before it
reached the Commission laboratory in Cairo on 10 September, where a part
of it was frozen in Dry Ice and the remainder lyophilized. These specimens
were then transported to the United States in Dry Ice, and after it was learned
that the subsequent clinical course of the patients from whom the blood was
drawn corresponded clinically to that of sandfly fever, the serum of two of
these was used for transmission tests in Cincinnati on 6 October 1943 (the
temperature charts of the two donor patients are shown in chart 5).

Each of four patients at the Longview State Hospital received 0.1 cc. of
serum intracutaneously and 0.75 cc. subcutaneously. All four of the recipients
developed typical sandfly fever after the usual incubation periods of 3 to 4 days
(for record of their temperatures, see "original subjects" in chart 6). These
four patients were in turn bled within a few hours after onset of fever, and the
resulting serum, stored in the frozen state in Dry Ice, constituted a fresh sup-
ply of virus for the identification of the agent responsible for the Sicilian
febrile illness. The cross-immunity tests by which the Sicilian agent was
identified are shown in charts 6 and 7. The first group of four subjects were
tested 1 month after recovery by reinoculation with homologous Sicilian ma-
terial. All four were found to be immune while four new human subjects,
inoculated simultaneously, developed the disease. Fifteen days later, these
original four subjects, who were thus proved to be immune to the homologous
Sicilian virus, were inoculated with the Middle East strain, whose identity as

8 Report, Maj. Albert B. Sabin, MC, to Chief Surgeon, Seventh U.S. Army, Col. Daniel Franklin,
MC, 7 Sept. 1943, subject: Estimate of Extent to Which Sandfly Fever Was and Is a Problem Among
American Forces in Sicily.
a sandfly fever virus had already been established by transmission tests with *P. papatasii*. All four were again found to be immune, while four controls inoculated simultaneously developed the disease. The reverse of this test, shown in chart 7, was carried out with patients who were inoculated first with the Middle East virus, and, after they were shown to be immune to the homologous Middle East agent, they were challenged with the Sicilian virus and also turned out to be immune. Thus, it was evident that approximately 6 weeks after an attack with one or the other virus, cross-immunity was readily demonstrable. It should be noted here that similar results were obtained in subsequent cross-immunity tests, which were carried out at 4 months as well as 2 years after a single attack, indicating that by active resistance tests the two agents were indistinguishable.

Properties of the Virus

**Infectivity of the virus by different routes.**—Using serum obtained within 24 hours after onset of fever and doses of 1 cc. or more, it was found that intracutaneous or intravenous routes of inoculation produced infection in approximately 95 percent of over 100 human adults regardless of sex or color.
Chart 6.—Cross-immunity between Sicilian and Middle East strains of phlebotomus fever virus

First inoculation
Sicilian virus
Original subjects

Second inoculation
Sicilian virus
Original subjects

Third inoculation
Middle East virus
Original subjects

Characteristic leukocyte changes

DAYS

Temperature -
During the course of experiments on filtrability of the virus, it was discovered quite by accident (when it was necessary to inject diluted serum in larger quantities by subcutaneous or intramuscular routes) that doses of virus which were infective by the intracutaneous or intravenous routes failed to produce the clinically apparent disease in 50 to 75 percent of individuals inoculated simultaneously by the subcutaneous or intramuscular routes. In two tests with the Sicilian virus, six of eight patients (75 percent) failed to develop the disease following subcutaneous or intramuscular inoculation, and in a single test with the Middle East virus, three of six patients (50 percent) failed to develop the disease following subcutaneous inoculation. The six individuals who failed to develop the disease following inoculation with the Sicilian virus were subsequently retested by the intracutaneous injection of a dose of virus which brought down all three controls, and all of the six again failed to show any signs of illness. Since it seems unlikely that so large a number would have been spontaneously resistant to begin with, it would appear possible that subcutaneous or intramuscular inoculation of the virus may have produced an inapparent infection with subsequent immunity.

Concentration of virus in infectious serum. The concentration of virus in serum obtained within a few hours after onset of the fever was measured (1) by determining the minimal dose which will produce the clinical disease in human volunteers, and (2) by reinoculating those volunteers who failed to show any signs of the disease to determine whether or not the subclinical doses produced immunity. The first experiment was done with a preparation of lyophilized serum which was obtained from a human volunteer in Egypt who developed the disease after being bitten by P. papatasii artificially infected in the laboratory. Of three patients inoculated with 0.1 cc. intracutaneously and 0.9 cc. subcutaneously, two developed the disease. However, none of nine other patients inoculated simultaneously with 0.1, 0.01, or 0.001 cc. (three patients for each dose) by the intracutaneous route developed the clinical disease. Upon subsequent challenge, all those who remained well after the initial inoculation were proved susceptible to larger doses of the virus. In a subsequent experiment with third passage Middle East virus, using the serum of a single patient, it was found that 0.01 cc. produced the typical disease in both inoculated patients; 0.001 cc. also produced a clinically recognizable illness in a single patient, while 0.0001 cc. failed to produce the disease in one patient. Although this represents a rather inadequate titration, it would appear that in this particular serum the minimum infective dose may have been 0.001 cc. However, in the third experiment with Middle East virus using seventh passage material from a single patient, the serum having been frozen in Dry Ice for 8 months, it was found that only one of four patients inoculated with 1 cc. intracutaneously developed the disease. A systematic titration was not carried out with the Sicilian strain of virus; however, of 15 individuals who received 1 cc. intracutaneously (virus represented by serum derived either from natural cases of the disease or from human subjects up to six experimental passages), 43 developed clinically apparent disease. All nine individuals who received 1.5
to 2 cc. of various passages of the Sicilian strain developed the clinical disease, and two of three patients who were inoculated with 0.5 cc. of Sicilian virus developed the disease. During the gradocol membrane filtration tests, part of the pool which was found to be infective with regularity in amounts of 1 cc. was also tested in smaller doses, two patients being inoculated with 0.0001 cc. and another two patients with 0.00001 cc., but none developed the illness. In subsequent tests for susceptibility by inoculation of larger amounts of the same virus, all four patients developed typical experimental sandfly fever. Thus, it can be said that the Sicilian virus also does not have a potency as high as 10,000 minimum infective doses per cubic centimeter of serum.

An interesting phenomenon which may represent a difference in the behavior of sandfly fever virus in American Negroes was encountered during the course of this work. Although it was found that Negroes were as susceptible as white people when inoculated with serum derived from white individuals with sandfly fever, it was not possible to obtain passage when the serum of Negroes with the disease was used. In three different experiments using 1 cc. amounts of serum from three different Negroes, negative results were obtained. When the volunteers used in these tests were subsequently inoculated with serum from white donors, all developed sandfly fever, indicating that they were susceptible. Since amounts larger than 1 cc. were not tested, one cannot say that the virus might not have been present in smaller concentration in the blood of Negroes who developed experimental sandfly fever. However, the results do suggest that the virus may perhaps not propagate to as high a level in Negroes as it does in white individuals. Since it had been demonstrated that the sandfly fever virus can be maintained in the lyophilized state or frozen in Dry Ice for a period of at least 4 years, it is not likely that the manner of storage of the serum seriously affected the results that were obtained.

**Particle size of the virus.**—Two experiments were carried out with the Sicilian strain of sandfly fever virus in an attempt to determine the particle size by means of filtration through gradocol membranes. Twenty-six human subjects were used for the tests, and the results are shown in table 23. The first test was unsatisfactory because the inoculations were given by the subcutaneous and intramuscular routes before it was realized that these routes were less suitable than the intracutaneous. However, the second experiment yielded clean-cut results in that all 10 volunteers inoculated intracutaneously and intravenously with either the diluted, unfiltered serum or the filtrates from the 600, 400, 310, and 207 mµ membranes developed typical sandfly fever, while the two patients inoculated with somewhat larger doses of the filtrate from the 101 mµ membrane both failed to develop any signs of illness or changes in the leukocytes. Although it is possible that one of these two patients may have had an inapparent infection since he failed to develop the disease on challenge with active virus later on, it is, nevertheless, evident that the average pore diameter of the endpoint membrane is in the range of 101 mµ. If one assumes that the 101 mµ membrane represents the filtration endpoint, the particle size of the virus may be estimated at 40 to 60 mµ, according to Elford’s
Table 23.—Filtration of sandfly fever virus through gradocol membranes

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Portion tested</th>
<th>Amount injected (cc.)</th>
<th>Patient</th>
<th>Result</th>
<th>Result of subsequent challenge with virus intracutaneously</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool of passage I serum, diluted 1:5 in saline, and centrifuged at 1,000 r.p.m. (all filtrates inoculated subcutaneously and intramuscularly),</td>
<td>Centrifuged, unfiltered...</td>
<td>5</td>
<td>D...</td>
<td>Negative...</td>
<td>Negative...</td>
</tr>
<tr>
<td></td>
<td>750 mg filtrate...</td>
<td>5</td>
<td>S...</td>
<td>do...</td>
<td>Do...</td>
</tr>
<tr>
<td></td>
<td>300 mg filtrate...</td>
<td>5</td>
<td>A...</td>
<td>Sandfly fever...</td>
<td>Sandfly fever...</td>
</tr>
<tr>
<td></td>
<td>200 mg filtrate...</td>
<td>5</td>
<td>B...</td>
<td>Negative...</td>
<td>Do...</td>
</tr>
<tr>
<td></td>
<td>100 mg filtrate...</td>
<td>5</td>
<td>M...</td>
<td>do...</td>
<td>Do...</td>
</tr>
<tr>
<td></td>
<td>75 mg filtrate...</td>
<td>5</td>
<td>K...</td>
<td>do...</td>
<td>Do...</td>
</tr>
<tr>
<td></td>
<td>50 mg filtrate...</td>
<td>10</td>
<td>H...</td>
<td>Negative...</td>
<td>Da...</td>
</tr>
<tr>
<td></td>
<td>Centrifuged, unfiltered...</td>
<td>2</td>
<td>i. cut.</td>
<td>C...</td>
<td>Sandfly fever...</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>i. ven.</td>
<td>CF...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>i. cut.</td>
<td>CL...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>i. ven.</td>
<td>P...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>i. cut.</td>
<td>BI...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>i. ven.</td>
<td>S...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>i. cut.</td>
<td>M...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>i. ven.</td>
<td>B...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>i. cut.</td>
<td>T...</td>
<td>do...</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>i. ven.</td>
<td>MI...</td>
<td>do...</td>
<td></td>
</tr>
</tbody>
</table>

1 Serum obtained from this patient at onset of fever produced typical sandfly fever in two other patients.

However, it must be kept in mind that the concentration of virus in the serum used for filtration was not very great. In addition to the controls shown in table 22, three other patients were each inoculated intracutaneously with 0.5 cc. of the same pool, and two of the three developed typical sandfly fever. However, four other subjects inoculated with 0.0001 cc. or 0.00001 cc. of the same pool failed to develop the disease and also failed to develop immunity to subsequent challenge. Thus, it would appear that the concentration of virus, although not precisely known, was certainly less than 10,000 M.I.D. (minimum infective dose) per cubic centimeter. Since it is known that the amount of virus contained in the material that is filtered can influence the filtration endpoint, one would have to conclude that while the particle size of the sandfly fever virus is probably not greater than 40 to 60 μ, there is a possibility that it may be smaller.

**Further tests in animals.**—During the course of the work with this virus in the United States, additional tests were carried out with sera of proved infectivity for human beings on the following animals: Infant mice, cotton rats,
hamsters, and monkeys. Because of the questionable results obtained in several hamsters in Egypt, suspensions of the brains and viscera of those hamsters, which had been stored in Dry Ice and transported from Egypt to the United States, were inoculated into six hamsters, but all remained well. An additional test on hamsters was carried out with the fourth human passage of the Sicilian strain of virus; the serum was injected in six young animals by the intracerebral, intracutaneous, intraperitoneal, and intratesticular routes, but all remained well. Mice were inoculated with undiluted serum, serum diluted 1:100, and also with the sediment from ultracentrifuged serum taken up in 1/50 of the original volume. Mice varying in age from 10 days to 4 weeks were inoculated by the intracerebral and the intraperitoneal routes, but all remained well. On several occasions, when mice presented questionable signs or died after a period of 13 or 14 days, passages were performed but with negative results. The sera which was used for these tests represented some which had had only one passage from the natural disease, as well as others which had had seven serial passages in human volunteers. Six cotton rats inoculated with first passage Sicilian virus by the intracerebral, subcutaneous, and intraperitoneal routes remained well. It should be noted, however, that in none of these experiments was material from any of the animals tested in human volunteers to determine whether or not the virus might have undergone inapparent multiplication.

In view of the fact that Shortt and his associates believed that they had transmitted the virus of sandfly fever to monkeys, a special experiment was undertaken with these animals. The Sicilian virus represented by a pool of passage I human serum was inoculated simultaneously into patients and into three rhesus monkeys. Each monkey received a total of 6 cc. of serum—one cc. by the intracerebral route, 0.1 cc. intracutaneously, 0.9 cc. subcutaneously, and 4 cc. intraperitoneally. None of the three monkeys developed fever or showed any other clinical evidence of disease. However, they were bled at different intervals, and the serum obtained 3 and 4 days after inoculation was subinoculated in two patients. It is noteworthy that while neither of the two patients exhibited anything that the experienced investigator could have interpreted as sandfly fever, each of them, nevertheless, developed a febrile illness, one on the 10th day and the other on the 11th day, associated with generalized malaise and joint pains. However, the leukocyte changes which are characteristic present in sandfly fever did not develop in either of these two patients. Clinically, it appeared more likely that the reaction in these patients was due to serum sickness resulting from the inoculation of the monkey serum. Approximately 4 weeks later, these patients were inoculated with sandfly fever virus and both of them developed typically severe forms of experimental sandfly fever, thus confirming the original clinical diagnosis of serum sickness and indicating that no virus was present in the blood of monkeys 3 and 4 days after inoculation of human sandfly fever virus. No evidence was, therefore, obtained that the sandfly fever virus was either pathogenic for rhesus monkeys or indeed multiplied inapparently during the period tested.
Although the work was halted because of the pressure of other investigations, it appeared that further experiments with newborn and 1- and 2-day-old rodents, particularly mice, would appear to be worthwhile not only in an attempt to establish the virus in a small experimental animal but more especially to determine whether the sandfly fever virus may be propagating inapparently. It would be desirable to test such material passaged in newborn rodents not only for pathogenicity in human beings but even more especially for the capacity to produce immunity to unmodified human virus should it turn out to be nonpathogenic.

Further cultivation attempts in embryonated eggs.—Although the experiments carried out in the Middle East laboratory indicated that no specific antigen suitable for complement fixation tests could be obtained from eggs inoculated with human serum containing sandfly fever virus, it was, nevertheless, desirable to determine whether or not inapparent multiplication of the virus may occur in embryonated eggs, as determined by subinoculation in human volunteers. The tests which were carried out in the United States are summarized in table 24. It may be seen that among the nine human volunteers inoculated with various types of chick embryo material, two developed febrile reactions 12 and 13 days after inoculation, but it was possible to show that in neither one of those instances was the fever due to infection with sandfly fever virus, because subsequent challenge produced the typical disease in both volunteers. Although in one test, two of four volunteers inoculated with fourth egg passage material failed to develop sandfly fever on challenge, it would appear more likely from the other results that these two human subjects might have represented individuals who were resistant to this virus to begin with. It was necessary to conclude from these tests that there was no evidence that the sandfly virus could multiply in chick embryos inoculated by the various routes that were tried. Accordingly, it was not possible to obtain confirmation of the conclusions reached by Shortt and his coworkers. It may also be stated here that an attempt was made to grow the sandfly fever virus in cultures containing minced mouse embryo brain or minced whole mouse embryo. Inoculation of the third passage culture material in human volunteers yielded negative results.

Further tests with A. aegypti.—Although a preliminary test carried out in the Middle East laboratory indicated that A. aegypti mosquitoes were unable to transmit the virus of sandfly fever, it was desirable to determine beyond doubt, as far as it may be possible to do so, whether A. aegypti mosquitoes in large numbers and after varying periods of extrinsic incubation can or cannot transmit this virus. In view of the hypothesis that had been forwarded by some investigators⁵⁰ that the sandfly fever and dengue viruses may belong to one group, it was particularly important to determine this point with great care. Three experiments were carried out with human volunteers in the United States, with the assistance of Lt. (later Capt.) William G. Jahnes, SnC. Large numbers of freshly emerged mosquitoes were allowed to feed on

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Table 24.—Human tests with chick embryo "cultures" initiated with sandfly fever virus of proved potency

<table>
<thead>
<tr>
<th>Inoculum for embryonated eggs</th>
<th>Site of inoculation, age of embryo, and passage</th>
<th>Material tested in human subjects</th>
<th>Subject</th>
<th>Result of inoculation</th>
<th>Result of subsequent challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle East strain, human passage III serum.</td>
<td>Embryo and yolk sac, 8-day eggs, passed at 4-day intervals.</td>
<td>4th egg passage, whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>K</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd egg passage, whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>B</td>
<td>Do</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd egg passage, whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>A</td>
<td>do</td>
<td>Sandfly fever. Do.</td>
</tr>
<tr>
<td>Department of Health.</td>
<td></td>
<td>3rd egg passage, whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>S</td>
<td>do</td>
<td>Do</td>
</tr>
<tr>
<td>Sicilian strain, pool of human passage V serum.</td>
<td></td>
<td>5th egg passage whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>F</td>
<td>4-day fever with pharyngitis plus leukocytosis 12 days after inoculation.</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5th egg passage whole embryo, 2 cc., 20 percent suspension, intracutaneous.</td>
<td>O</td>
<td>Negative</td>
<td>Do</td>
</tr>
<tr>
<td>Sicilian strain, pool of human passage VII serum.</td>
<td></td>
<td>3rd passage, chorio-allantoic membrane suspension, 1 cc.</td>
<td>Bo</td>
<td>1-day fever 13 days after inoculation; no leukocyte changes.</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd passage, chorio-allantoic membrane suspension, 1 cc.</td>
<td>Su</td>
<td>Negative</td>
<td>Do</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd passage, chorio-allantoic membrane suspension, 1 cc.</td>
<td>M</td>
<td>da</td>
<td>Not tested.</td>
</tr>
</tbody>
</table>

¹ Two controls inoculated simultaneously developed the disease.

human volunteers during the first 24 hours after onset of fever during the experimental disease. At the time the mosquitoes fed on these volunteers, blood was taken in each instance to determine, by subinoculation in other volunteers, that infective virus was actually present at the time of the biting. One experiment was carried out with the Middle East virus and two tests were carried out with the Sicilian strain of the virus. Mosquitoes which had taken blood meals from infected individuals were allowed to bite new volunteers after extrinsic incubation periods varying from 6 to 22 days. Six human volunteers were bitten by hundreds of mosquitoes which had taken blood meals from infected human beings at the time when their blood was shown to contain the virus, but none of them developed sandfly fever. The susceptibility of all these volunteers was subsequently demonstrated when they developed the typical experimental disease following inoculation of serum from the very donors on which the mosquitoes had obtained their blood meals. This unequivocal demonstration of the inability of A. aegypti to act as a vector of the virus of sandfly fever became an important tool in the differentiation of this virus from that of dengue.

Tests for virus in cerebrospinal fluid and in blood.—Observations on more than 100 cases of the experimentally produced disease indicated that the duration of fever in different individuals may vary from part of 1 day to 9 days, although the 2-, 3-, and 4-day periods constituted 85 percent of the total (chart 8). Multiple cycles of fever (chart 9) after a single inoculation of virus were observed in four patients. Subinoculation experiments made it possible to show that the virus was present in the blood 24 hours before the
onset of the fever but could not be demonstrated in the blood of several patients 48 to 54 hours after onset of the fever. No explanation was found for the multiple cycles of fever. Since no virus could be recovered from the blood during the periods of recurring fever, the relapses could not be explained on the basis of recurring viremia.

Cerebrospinal fluid was obtained from five of the American volunteers during the work in Egypt. No pleocytosis or other abnormalities were found in those fluids, and part of each fluid was lyophilized and the remainder frozen in Dry Ice and transported to the United States. Two patients were inoculated with the pool of these cerebrospinal fluids, each receiving 2 cc. intracutaneously and 15 cc. intravenously. Neither developed the fever or the leukocyte changes characteristic of sandfly fever, and both were subsequently
shown to be susceptible to an inoculation of the virus. It is noteworthy that the serum of the same volunteers, lyophilized and frozen in the same manner and transported to the United States, was also tested on human volunteers and proved to be infective.

Storage and maintenance of infectivity of the virus. Since so much work was involved in establishing the identity of an authentic strain of the virus of sandfly fever, it was of considerable importance to determine whether or not and under what conditions it may be possible to preserve this virus. Accordingly, at various intervals, portions of human serum maintained in the frozen state in Dry Ice, as well as portions of serum which had been lyophilized and then stored in an ordinary refrigerator, were tested in human volunteers. When the work on sandfly fever was terminated at the end of 1945, it was found that the second passage pool of the Sicilian strain, which was frozen in Dry Ice on 30 November 1943, still produced the disease in both patients receiving 2 cc. of the serum intracutaneously on 10 October 1945. Thus, it was clear that the virus persisted for at least 2 years in that state. Lyophilized virus tested at the end of 6 months was found to be active. In subsequent years, an opportunity presented itself to test some of the lyophilized and frozen virus on children with nephrosis as part of a trial therapeutic study, and it was possible to show that the virus remained active after a period of storage of at least 5 years. These strains of virus are still available in the author’s laboratory in both the frozen and the lyophilized states. It is hoped that virus preserved in this manner may prove useful for any future comparative purposes or studies that may be contemplated.

Tests for neutralizing antibodies. Determination of the presence or absence of neutralizing antibodies in sandfly fever was desirable not only for academic reasons but also (1) to find a serological means for the identification of the virus or its various types, and (2) to establish whether or not active immunity could be produced by the inoculation of neutral serum-virus mixtures. Two tests were carried out, and the results are summarized in table 25. The hyperimmune serum used in these tests was derived from four patients who recovered from an experimental attack of the disease following inoculation of the Sicilian strain of the virus. Four weeks later, they were reinoculated with the Sicilian strain and were found to be immune. Two weeks after that, they received an inoculation of the Middle East strain of the virus of proved potency, and they were again resistant. Two weeks after this second challenge, or 8 weeks after the first inoculation of virus, they were all bled, and their serum constituted the hyperimmune serum used in these tests. These results shown in table 25 indicate that in neither test was complete neutralization of the virus obtained. It is possible, however, that a certain degree of neutralization did occur, because in each of the tests there was one subject who failed to develop any signs of illness or leukocyte changes following inoculation of the immune serum-virus mixtures and yet, on challenge, subsequently developed typical sandfly fever. These challenge tests indicated not only that the subjects were originally susceptible but, also, incidentally, that the inoculation of the hyper-
immune serum-virus mixture does not lead to the development of active resistance to the virus.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Strain of virus</th>
<th>Mixture inoculated</th>
<th>Subject</th>
<th>Result</th>
<th>Result of subsequent challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Middle East</td>
<td>Virus only, 1 cc...</td>
<td>1</td>
<td>Sandfly fever</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus (1 cc.) plus hyper-immune serum (1 cc.) 1.5 hours at 23° C.</td>
<td>2</td>
<td>do</td>
<td>Sandfly fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus only, 0.5 cc.</td>
<td>3</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus (0.5 cc.) plus hyper-immune serum (1 cc.) 2.3 hours at 23° C.</td>
<td>4</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sicilian</td>
<td>Virus only, 1 cc...</td>
<td>5</td>
<td>Sandfly fever</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus (1 cc.) plus hyper-immune serum (1 cc.) 1.5 hours at 23° C.</td>
<td>6</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus only, 0.5 cc.</td>
<td>7</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virus (0.5 cc.) plus hyper-immune serum (1 cc.) 2.3 hours at 23° C.</td>
<td>8</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>do</td>
<td>Negative</td>
</tr>
</tbody>
</table>

PERSISTENCE OF IMMUNITY FOLLOWING A SINGLE ATTACK OF SANDFLY FEVER

Various observations recorded in the literature concerning persistence of immunity to sandfly fever and recurrence of the disease seemed to be confusing and contradictory. On the one hand, there are reports of multiple attacks, occasionally also during one season, and on the other, there are numerous observations that so-called “salted” troops (that is, troops that had once experienced an epidemic of sandfly fever) proved to be resistant during subsequent seasons, at a time when newly arrived troops were contracting the disease. Whether or not a single attack of the disease in residents in a sandfly fever area had to be fortified by repeated exposures to the virus in order to acquire lasting immunity was not known. The studies which were carried out in the United States, a country free of the disease, made it possible to determine the immunogenic effect of a single experimental attack of sandfly fever when the same strain of virus, or an immunologically closely related or identical strain, was used for challenge.

The data summarized in table 26 indicate that the 8 volunteers tested 1 month after infection and the 10 volunteers tested 4 months after infection all remained well, while control patients inoculated simultaneously with the same virus developed the disease. In a test carried out 2 years after a single attack of the disease, three of the four patients remained entirely well, showing neither fever, symptoms, nor changes in the leukocytes, while the fourth patient may have experienced a very mild, modified form of the infection. Although this patient (Mc) had no subjective complaints, he, nevertheless, exhibited a rise in temperature of 1° F. for a period of about 24 hours 6 days after inoculation. This was associated with changes in the leukocytes characteristic of this infection. It is regrettable that the presence of reinfection in this individual
was not proved or disproved by transmission of the disease to others. However, it would appear that in the majority of individuals immunity to reinfection with the same immunologic type of sandfly fever virus was present for a period of at least 2 years. If the events which occurred in the fourth patient tested at 2 years were, in effect, due to reinfection it may perhaps indicate that as immunity wanes reinforcement might be possible by essentially subclinical infections.

Tests on two American physicians who had resided in Palestine for varying periods yielded interesting results with reference to the persistence of immunity. Both of them had lived in Cincinnati before 1932, and both gave histories of two previous attacks of the disease during a period of residence in Palestine.

Dr. Helen Glueck had her first attack during the summer of 1932, 5 days after she landed in Palestine. She returned to the United States in 1934 and lived there until 1937 when she again went to Palestine. After a trip to Syria in August 1937, she experienced a febrile illness of 2 days' duration associated with leukopenia which was again diagnosed as sandfly fever; that is, a second attack of the disease after an interval of 5 years. She returned to the United States in 1939 and volunteered for an immunity test in 1943; that is, 6 years after the presumable second attack of sandfly fever. She was inoculated with the Middle East strain of virus (1 cc. of passage III human serum intracutaneously) and after an incubation period of 3 days developed a typical, moderately severe attack of sandfly fever, associated with characteristic leukocyte changes.

Dr. Irwin Dunsky experienced his first attack of sandfly fever in Jerusalem during the summer of 1934, and another similar attack, of somewhat diminished severity, during the summer of 1935. He returned to the United States in 1936, where he resided continuously until he volunteered for an immunity test in November 1943; that is, 8 years after the last presumable attack. He received the same virus and dose which was administered on the same day to Dr. Glueck and three previously uninoculated patients. Three days after inoculation, he became mildly ill (aching in the neck, "giddy," and mild intermittent abdominal distress) for a period of about 4 hours during which time his temperature was 1.5° F. above his normal level for that time of day. Although the total number of leukocytes dropped from 6,900 to 4,400, there was no associated change in the differential formula.

The history of these two physicians shows how difficult it is to interpret the significance of presumably repeated attacks in individual patients, when one is dealing with a disease for which there is no specific clinical identifying sign or laboratory test. Thus, one is faced with several possible explanations. It is possible (1) that one or both attacks of the natural disease diagnosed as sandfly fever were not sandfly fever, (2) that immunity to sandfly fever may not persist, or (3) that multiple immunologic types of the virus may be involved.
Table 26.—Persistence of immunity to homologous type of sandfly fever virus in human subjects residing in the United States.

<table>
<thead>
<tr>
<th>Interval between experimental attack and challenge</th>
<th>Virus used for challenge</th>
<th>Virus previously inoculated</th>
<th>Subject</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>Sicilian, passage I, 1 cc., intraocular.</td>
<td>None</td>
<td>J</td>
<td>Sandfly fever.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>Remained well.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>W</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ri</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle East, passage II, 1 cc., intraocular.</td>
<td>None</td>
<td>Me</td>
<td>Sandfly fever.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>No fever, but characteristic leukocyte changes and symptoms.</td>
<td></td>
</tr>
<tr>
<td>4 months</td>
<td>Sicilian, passage V, 0.5 cc., for controls, 1 cc., for convalescents.</td>
<td>None</td>
<td>Ca</td>
<td>Remained well.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wo</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Br</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Im</td>
<td>Sandfly fever.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ro</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sicilian</td>
<td>A</td>
<td>Remained well.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mu</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>V</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ra</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle East</td>
<td>D</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wa</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Me</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>Sandfly fever.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mea</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>Sicilian, passage II, 2 cc., intraocular.</td>
<td>None</td>
<td>O</td>
<td>Remained well.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sicilian</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle East</td>
<td>Me</td>
<td>Modified sandfly (?) 1° F. above normal for 24 hours, 6 days after inoculation; associated with characteristic leukocyte changes.</td>
</tr>
</tbody>
</table>
LACK OF IMMUNOLOGIC RELATIONSHIP BETWEEN SANDFLY FEVER AND DENQUE

There was a tendency on the part of Van Rooyen and Rhodes to regard dengue, sandfly fever, and yellow fever as belonging to one group of agents. It had already been demonstrated in the present studies that sandfly fever virus could not be transmitted by A. aegypti and, thus, differed very definitely from the viruses of dengue and yellow fever. Tests recorded in table 27 show that not even partial cross-immunity could be demonstrated between the viruses of dengue and sandfly fever. Patients who had recovered from infections with either the Middle East or the Sicilian strain of sandfly fever virus developed typical, unmodified dengue following inoculation of human dengue serum. Similarly, the disease which following inoculation with sandfly fever virus was in no way modified when it occurred in an individual who had recovered from dengue.

<table>
<thead>
<tr>
<th>Virus inoculated</th>
<th>Virus previously inoculated</th>
<th>Interval (weeks)</th>
<th>Subject</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue, Hawaii (from natural cases)</td>
<td>None</td>
<td>10</td>
<td>S</td>
<td>Typical dengue.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>T</td>
<td>Do.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>L</td>
<td>Do.</td>
</tr>
<tr>
<td>Middle East sandfly</td>
<td>10</td>
<td>W</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>Sicilian sandfly</td>
<td>20</td>
<td>C</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>Sandfly, Sicilian</td>
<td>18</td>
<td>H</td>
<td>Typical sandfly.</td>
<td></td>
</tr>
</tbody>
</table>

IMMUNOGENIC ACTIVITY OF VIRUS IRRADIATED WITH ULTRAVIOLET LIGHT

As regards the attempts to produce active immunity in susceptible human beings, the results described thus far indicate (1) that subinfective doses inoculated intraeutaneously or intravenously failed to produce immunity to larger amounts of virus; (2) that while the intramuscular and subcutaneous injection of infective amounts of virus seemingly frequently led to the development of an inapparent infection with subsequent immunity, this procedure was impracticable since approximately 50 percent of those inoculated might develop the disease; and (3) that virus passaged in chick embryos or mouse-embryo cultures failed to produce the disease and also failed to produce immunity. Accordingly, it was of particular interest to determine whether or not inactivation of the virus by some mild means, such as irradiation with ultraviolet
light, might abolish its infectivity without loss of immunogenic capacity. The results of the tests with the Middle East and Sicilian strains of the virus on 26 patients are shown in table 28. It may be seen that with the particular apparatus used for irradiation the effect on infectivity was highly irregular. Thus, in the experiment with the Middle East virus, all four patients inoculated with the unirradiated virus developed sandfly fever, while five of the six patients inoculated with the same material irradiated for either 15 or 30 minutes failed to develop the disease. The one individual who did develop sandfly fever was inoculated with serum irradiated for 30 minutes. In the experiment with the Sicilian virus, 4 of 5 patients inoculated with the unirradiated virus developed the disease, while only 7 of 13 inoculated with the same serum irradiated for either 15, 35, or 60 minutes developed the disease. It is worth noting that increasing the time of irradiation up to 60 minutes failed to abolish the

<table>
<thead>
<tr>
<th>Strain of virus</th>
<th>Experiment date</th>
<th>Time of irradiation (minutes)</th>
<th>Inoculum</th>
<th>Subject</th>
<th>Result</th>
<th>Subject of subsequent challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle East pool of human passage V sera.</td>
<td>24 Nov. 1943</td>
<td>0</td>
<td>1 cc., intracutaneous.</td>
<td>E</td>
<td>Sandfly fever</td>
<td>Negative, Da, Da.</td>
</tr>
<tr>
<td>Sicilian pool of human passage III sera.</td>
<td>24 Jan. 1944</td>
<td>35</td>
<td>0</td>
<td>do.</td>
<td>do.</td>
<td></td>
</tr>
</tbody>
</table>

*In the Sicilian virus group, five controls inoculated simultaneously with the same dose of virus given to the seven subjects (Sa, H, D, Co, H.V., P, and Gre) for challenge, all developed typical sandfly fever; in the Middle East group, of four controls inoculated simultaneously with the same dose of virus given to the five subjects (M, L, Mm, A, S), three developed typical sandfly fever.*
infectivity. However, of the 11 individuals inoculated with the irradiated serum who failed to develop the disease, all were resistant on subsequent challenge with virus of proved infectivity in simultaneous tests. Since subinfective amounts of unirradiated virus were not found to be capable of producing immunity, and since it seems improbable that 11 resistant individuals were encountered in these tests, it would appear that after irradiation by ultraviolet light for a period insufficient completely to destroy the virus, subinfective amounts may, nevertheless, be immunogenic. It was intended to repeat these experiments during the latter part of 1944, using a different machine for ultraviolet irradiation, but the difficulty was encountered that the serum obtained from volunteers who developed typical sandfly fever proved to be noninfectious in amounts of 1 cc. It was not realized until considerably later that the use of Negro volunteers was probably responsible for yielding serum which either was of a very low infectivity or was completely noninfective. As matters stand now, no conclusive results are available, but the data are sufficiently interesting to warrant repetition.

STUDIES ON AN IMMUNOLOGICALLY DISTINCT SANDFLY FEVERLIKE VIRUS IN NAPLES, 1944

The incidence of sandfly fever among U.S. troops in Italy in 1944 (pp. 120–122) was high. In connection with a study carried out by Maj. Marshall Hertig, SnC, and Lt. Col. Ross L. Gauld, MC, of an outbreak among Allied Force Headquarters personnel, a number of sera were collected in July 1944, by Capt. (later Maj.) Frederick C. Robbins, MC, 15th Medical General Laboratory, at the suggestion of Col. William S. Stone, MC, NATUSA (North African Theater of Operations, U.S. Army). The clinical manifestations exhibited by all these patients were as follows:

1. Abrupt onset.
2. Temperatures high, in some cases 105°F, lasting from 1 to 5 days, most commonly 3 days.
3. Aches and pains in muscles with severe headache and often pain in the eyes on pressure or movement.
4. Diffusely injected conjunctivae.
5. Low white counts averaging 3,000 with tendency to lymphocytosis.
6. Complete recovery with only symptomatic therapy.

Major Hertig reported that P. papalasii was practically the only sandfly found in the building occupied by these people. The sera, which were stored in Dry Ice from the time they were collected, ultimately reached the United States and after a number of months were used for passage in human volunteers at the New Jersey State Prison in Trenton.

The serum of one patient was selected for trial because it was obtained within 24 hours after onset of his fever. It was collected on 12 July 1944, and tested on 18 December 1944; 1 cc. of this serum was injected intracutaneously
in five different sites, and 4.4 cc. were given intravenously to one human volunteer. After an incubation period of 3 days, this volunteer developed a 3-day fever associated with the same type of symptoms which were exhibited by the patients in Naples (that is, those ordinarily found in sandfly fever), and this was associated with a change in the leukocytes also characteristically found in sandfly fever or dengue. Blood was obtained from this volunteer within 24 hours after onset of his symptoms and fever, and \textit{A. aegypti} mosquitoes were allowed to feed on him at that time. The serum from this volunteer was subsequently inoculated, during the course of various experiments, in nine other volunteers and reproduced the same disease. The incubation period was, in all instances, short, usually 3 days, and in no instance was any lesion produced locally at the site of inoculation, nor did rash occur in any of the volunteers.

Two volunteers who had recovered from typical attacks following infection with the Sicilian strain of sandfly fever virus 6 weeks and 11 weeks before, respectively, were not immune upon inoculation with this Naples strain of virus. Their disease was in no way different from that of the controls nor from their original attack of sandfly fever. Two other volunteers, who had recovered from typical, proved infections with the Hawaii strain of dengue virus, 6 weeks and 10 weeks before, respectively, developed unmodified attacks of the disease following inoculation with the Naples strain of virus. Large numbers of \textit{A. aegypti} mosquitoes which fed on two human volunteers within 24 hours after onset of their fever were subsequently allowed to bite two other volunteers after prolonged periods of extrinsic incubation. One of the volunteers was bitten by a total of 93 \textit{A. aegypti} mosquitoes (13 after an 18-day extrinsic incubation period, 30 after a 29-day extrinsic incubation period, 24 after a 21-day incubation period, and 26 after a 32-day incubation period). The other volunteer was bitten by a total of 84 mosquitoes (20 within a 13-day extrinsic incubation period, 33 within a 17-day extrinsic incubation period, and 31 within a 24-day extrinsic incubation period). Both of these volunteers remained entirely well and 3 to 4 weeks after this exposure were inoculated with the Naples virus infectious serum. Both developed the typical experimental disease indicating that they were susceptible to this virus and that the \textit{A. aegypti} mosquitoes could not act as a vector of this agent.

Thus, we had evidence of an agent which was capable of reproducing a disease simulating sandfly fever in human beings, incapable of being transmitted by \textit{A. aegypti}, but at the same time immunologically unrelated to both the dengue and the sandfly fever viruses. In order to determine whether or not the lack of immunologic relationship or cross-immunity with sandfly fever virus worked both ways, two volunteers who had recovered from infection with the Naples virus were reinoculated with the same virus 1 month later. They resisted this second inoculation although other volunteers inoculated simultaneously developed the disease in the usual fashion. These two volunteers, thus shown to be resistant to the Naples virus, were then inoculated with
the Sicilian strain of sandfly fever virus and proceeded to develop after the usual incubation period a typical attack of sandfly fever. Accordingly, it was proved that the Naples virus, which was capable of giving rise to immunity to itself, was incapable of producing resistance to the only available type of sandfly fever virus. Conversely, the Sicilian-Middle East type of sandfly fever virus capable of giving rise to immunity to itself was incapable of inducing resistance to the Naples virus. The only way to prove that the Naples virus was indeed a sandfly fever virus would have been to establish the fact of its transmissibility by P. papatasii. Since P. papatasii could not be imported into the United States, and since it was not feasible to carry out such tests at that time elsewhere, a gradocel membrane filtration test was carried out to determine whether the particle size of this unknown Naples strain would be similar to or different from that of the Sicilian-Middle East type of sandfly fever virus. Since the number of volunteers available for this work at that time was already limited, a small test was carried out. Serum of known infectious potency diluted 1:4 in physiologic salt solution was serially put through gradocel membranes having an average pore diameter of 770, 600, 400, 270, 101, and 75 m\(\mu\). The filtrates from the 400, 270, 101, and 75 m\(\mu\) membranes were each injected into one volunteer intravenously in amounts of 10, 11, 16, and 7.5 cc., respectively. The volunteers who received the 400 and 270 m\(\mu\) filtrates each developed the typical experimental disease, while those who received the 101 and 75 m\(\mu\) filtrates remained well. Accordingly, even in this limited test, it was found that the filtration endpoint of the Naples virus was identical with that previously obtained with the Sicilian strain of sandfly fever virus. Thus, even though it proved impossible to carry out the definitive test of transmission by P. papatasii, the available laboratory and circumstantial epidemiologic evidence suggest that the Naples strain of virus in all probability is a strain of sandfly fever virus which is immunologically distinct from the Sicilian-Middle East variety.\(^5\) It has, therefore, been demonstrated for the first time that multiple immunologic types of sandfly fever virus might exist, which could explain the reports of multiple attacks in one season.

Only limited tests with the Naples strain of virus were carried out in laboratory animals. Serum freshly obtained from human volunteers within 24 hours after onset of their fever was inoculated intracerebrally into 2-week-old Swiss mice. Of the 18 mice inoculated with human serum, 4 developed rather definite signs of central nervous system disturbance 5 to 6 days after inoculation, and 3 of these 4 mice died. However, passage of 2 of these mice into 28 others yielded completely negative results. This work had to be discontinued before it could be determined whether or not inapparent multiplication of the Naples virus occurred in the brains of mice.

CONCLUSIONS

In conclusion, it may be said that while the laboratory investigations on the virus or viruses of sandfly fever, which were encountered in the Mediterranean area and in the Middle East, gave us a good deal more information on the basic properties of the virus and, also, provided a supply for storage and future studies, the primary objectives of developing some method of propagating the virus outside the human body and of a vaccine for the protection of exposed personnel were not attained. It is, therefore, particularly fortunate that DDT was found to be so highly effective for the control of P. papatasii in dwellings and that the available mosquito repellents proved so effective in protecting those who may be exposed out of doors. Even if future efforts should not lead to the discovery of a vaccine, sandfly fever need no longer be the military problem that it had been in various operations in endemic areas in the past.

FIELD OBSERVATIONS ON SANDFLY FEVER IN AMERICAN FORCES WITH SPECIAL REFERENCE TO PERIOD OF COMBAT IN SICILY IN 1943

Albert B. Sabin, M.D.

The field observations to be related in this section in the history of sandfly fever during World War II are intended to illustrate the following points:

1. The sources of confusion resulting from unfamiliarity with the disease.
2. The inadequacy of official statistics on this disease.
3. The unfortunate results of treating all F.U.O.'s as potential cases of malaria under the pressure of combat conditions in a region where sandfly fever is endemic.
4. The importance of this disease under combat conditions in Sicily in 1943.
5. The failure of putting to practical use the available information on insect repellents.

Early Experiences

In June and July 1943, two epidemics of sandfly fever were observed by members of the Commission on Neurotropic Virus Diseases, Army Epidemiological Board, stationed in the Middle East, the first being at Deversoir Field near the Great Bitter Lake on the Suez Canal, and the second at Camp Atterbury in Teheran (Iran) (p. 134). At Deversoir Field, the epidemic was 2 months old before its real nature became apparent to the medical officers attached to the installation. The presence of P. papatasii and of an illness compatible with sandfly fever was confirmed by members of the Commission, and Dr. John K. Paul, Director of the Commission, made a personal examination of the dispensary records from January to December of 1943 and graphically summarized the data shown here as chart 10. It is noteworthy that in the beginning sandfly fever was commonly diagnosed as influenza. While the secondary increase in the number of cases during September and October is associated with cessation of isolation of patients, it is not known to what extent newly arrived personnel or an increase in the number of sandflies or a change in climatic conditions may have been involved. Nevertheless, it is known that patients, who are not isolated or otherwise protected from sandflies, may serve to increase the infection rate among sandflies. During the first month of the epidemic in Teheran, the cases were listed and treated as malaria.
Note.—Arrow at point A indicates date on which "isolation" of cases was begun and at point B on which it was stopped. Each small square represents one case.
Clinical and Epidemiologic Survey in Sicily

In view of these earlier experiences, it appeared desirable to estimate, if possible, the extent to which sandfly fever was a problem during the period of active military operations in Sicily. The presence of *P. papatasii* and *P. perniciosus* on the island was previously recorded and confirmed in August 1943, by Maj. (later Col.) Cornelius B. Philip, SnC, the entomologist of the Commission on Neurotropic Virus Diseases in the Middle East. The clinical and epidemiologic survey was made by this author during the first week of September 1943, and most of what follows is taken from a report which he submitted to Col. Daniel Franklin, Surgeon, Seventh U.S. Army, at that time and from supplementary data contained in his diary and files.

The extent to which American troops were being affected by sandfly fever was estimated according to two lines of thought: First, by comparison of the clinical manifestations and course of "fevers" observed in evacuation and field hospitals with those of known sandfly fever (the medical officer making this comparison and submitting this report had just spent 4 months in the Middle East observing this disease as it occurs in troops and studying the experimental disease as it was reproduced in American volunteers); second, by determination of the probably relative proportion of malaria and the syndrome corresponding to sandfly fever on the basis of the clinical course and manifestations of a large sample of "fevers" unaffected by the early administration of the routine course of antimalarial therapy.

Extensive rounds were made at the 59th and 91st Evacuation Hospitals and at the 11th Field Hospital, where large numbers of patients diagnosed as malaria, F.U.O., and sandfly fever were examined and questioned. The findings were as follows:

**59th Evacuation Hospital.**—Cases then in the hospital diagnosed as pappataci fever had not received the routine course of antimalarial therapy and presented a syndrome entirely compatible with that disease.

Cases of fever were seen in the first and second days of the disease whose symptoms suggested pappataci fever and were not submitted to the course of antimalarial therapy.

The term "F.U.O." in this hospital was reserved for cases with negative malarial smears and clinical histories which suggested to them neither malaria nor pappataci fever, nor any other diagnosis. These were untreated, except for codeine and aspirin, and recovered spontaneously within a few days. My examination of such patients on the wards, as well as of the clinical records of those that had been discharged, led me to the belief that practically all of these patients presented syndromes compatible with the various manifestations of sandfly fever.

As regards malaria, the records of this hospital are confused by the fact that when the hospital first arrived on the island, there had been evacuated to it a large number of patients with the diagnosis of malaria on whom routine antimalarial therapy had already been started. It was believed, however, that many of these patients did not have malaria and that in many instances the reports of positive smears which came with those patients could not be relied upon. Among the patients now on the wards, however, it was striking how the histories of those diagnosed as malaria, whether or not the smear was positive, differed significantly from those diagnosed as pappataci fever or F.U.O.

Because standard criteria were not employed by all the medical officers in this hospital, it was not possible to use the registrar's report for a reliable estimate of the proportion of probable malaria and sandfly fever. Among the patients on the wards, there were at least as many with the diagnoses F.U.O. and pappataci fever as there were with malaria.

**91st Evacuation Hospital.**—This hospital presented, in my opinion, the best opportunity for estimating the relative proportion of sandfly fever and malaria occurring in at least one part of Sicily, for the following reasons:

1. After approximately the first 3 weeks in Palermo (27 July to about 15 August 1943) routine antimalarial therapy was no longer started immediately on all fevers. It was not administered until the clinical course, with or without positive smear, suggested malaria
Thus, it was observed that a large number of the fevers defervesced spontaneously and presented a clinical syndrome entirely compatible with sandfly fever.

2. The medical officers had decided to use standard criteria for diagnosis of pappataci fever and malaria. F.U.O. was used only on the wards until the clinical course of the case became clear, and it practically disappeared as a discharge diagnosis from the registrar's records.

3. The registrar's statistics for the period of 14 August to 3 September are based on the criteria just mentioned.

On one ward of 52 patients, there were 16 with typical histories and courses of pappataci fever unaffected by antimalarial therapy and perhaps 4 to 6 more still diagnosed as F.U.O.

At my request, the registrar, Capt. Stewart C. Wagoner, MC, prepared the statistics separately for the first period (27 July to 13 August) and the second period (14 August to 3 September). These statistics are shown in table 29 and throw the best light on the proportion of probable malaria to probable sandfly fever.

The statistics of the first period are not unlike those of the 59th Evacuation Hospital, while those of the second period present perhaps the most accurate picture of what occurred among a group of 922 cases of “fever” (excluding dysentery and diarrhea). Approximately 69 percent of these (637 cases) could be considered as pappataci fever—their clinical course was compatible with that disease; they recovered promptly without antimalarial therapy, and their malaria smears were negative.

During the second period, the probable sandfly fever cases constituted 58.6 percent (637 of 1,087) of the total communicable diseases and 33.3 percent (637 of 1,914) of all the admissions to the 91st Evacuation Hospital.

11th Field Hospital.—The situation in this hospital was still the same as that which prevailed everywhere during the early weeks of the campaign: that is, practically all patients with fever except those with obvious dysentery were put on the routine course of antimalarial therapy.

The medical officers had heard of sandfly fever but did not attempt to make the diagnosis.

<table>
<thead>
<tr>
<th>Table 29. Admission, classification, and discharge diagnosis, 91st Evacuation Hospital, Palermo, Sicily, 27 July–3 September 1944</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification or discharge diagnosis</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Battle casualties</td>
</tr>
<tr>
<td>Injuries</td>
</tr>
<tr>
<td>All diseases</td>
</tr>
<tr>
<td>Total admissions</td>
</tr>
<tr>
<td>Dysentery</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Pappataci fever</td>
</tr>
<tr>
<td>Fever of undetermined origin</td>
</tr>
<tr>
<td>Malaria:</td>
</tr>
<tr>
<td>Unclassified (positive smear)</td>
</tr>
<tr>
<td>Unclassified (negative smear, diagnosed clinically)</td>
</tr>
<tr>
<td>Tertian</td>
</tr>
<tr>
<td>Estivo-automnal</td>
</tr>
<tr>
<td>Total communicable diseases</td>
</tr>
</tbody>
</table>

Note.—After about 14 August 1943, malarial therapy was no longer administered routinely to all “fevers.”
Of four new patients with negative malaria smears whom I examined and questioned, three gave rather typical stories for beginning sandfly fever (these would have been observed at the 91st Evacuation Hospital but were immediately started on antimalarial therapy here) and one a similarly suggestive story of malaria—a history of recurrent attacks of fever and spontaneous defervescence over a period of 5 days with a temperature of 98.4° F. on admission.

Two patients who had positive smears and had been receiving the course of antimalarial therapy for a number of days were examined and questioned. One gave a typical history of malaria; that is, recurrent chills and fever before treatment and for a period of 3 days after quinine. The other, however, gave a common story of sandfly fever with a temperature of 101° F. on the first day, 100° on the second day, and 99° on the third day of his illness.

One patient who was said to have a positive smear on admission presented the most characteristic manifestations and course of infectious hepatitis.

The problems presented by some of these patients are (1) the occurrence of other febrile diseases among individuals with positive malaria smears on suppressive therapy, and (2) the number of smears that may erroneously be called positive in the field.

Estimated Incidence

An estimate of the extent to which the fevers (malaria and pappataci) were a problem during the campaign was obtained from interviews with the commanding officer of the 56th Medical Battalion and the surgeon of the 3d Infantry Division of the Seventh U.S. Army, and from the statistical compilation which was made available by the Office of the Surgeon, Seventh U.S. Army.

From the 56th Medical Battalion, it was learned that, during the first 10 days of the campaign, fevers were not a problem and that casualties constituted the major part of their work. After 22 July, however, the impression was that casualties made up only 10 percent of their work and the fevers most of the remainder. Antimalarial therapy was started on practically all fevers, and because the patients were quickly evacuated, there was no opportunity to reach any final conclusion on the nature of the disease in most instances. The impression was that many of the smears were probably erroneously called positive and that at least a certain number of untreated troops had self-limited, short (2- to 4-day) fevers incapacitating them for 5 to 7 days.

From the 3d Infantry Division, it was also learned that the fevers did not become a problem until after 21 July when the division entered and bivouacked in Palermo. During the second part of their campaign, 1-17 August, with a total strength of 18,814, they had 1,257 cases of disease, 201 of injury, and 1,055 of wounded. Although the division adopted a rigid Atabrine (quinacrine hydrochloride) discipline after 23 July (0.1 gm. being taken daily), the cases of fevers continued to mount. However, very few men were lost to the division as a result of fevers. The majority were evacuated to the field hospital and to the evacuation hospital which followed the division and were returned to duty in 4 to 10 days. The percentage sick in hospital at any one time was not more than 1 percent, and the function of the division was said not to have been affected seriously.

The statistics for disease reported for the entire Seventh U.S. Army during the period of 10 July 1943 (D-day) to 3 September 1943 (table 30) listed only 248 cases of sandfly (pappataci) fever, but at the same time, there were 6,862 cases of F.U.O. and 7,382 cases of malaria, of which only 4,831 had positive smears. The combined incidence of sandfly fever, F.U.O., and malaria was 14,492. If the same proportion (87.6 percent) of sandfly fever which was found among the 322 carefully observed patients with this group of "fevers" at the 91st Evacuation Hospital is applied to the fever cases (9,661, excluding the 4,831 malaria cases reported to have had positive smears) for the entire Seventh U.S. Army, for the reported period, it is estimated that approximately 8,500 may have been