

A SCHOLARSHIP plan for the encouragement of promising young scientific students has been adopted by the Radio Corporation of America. The plan provides for ten students to receive RCA scholarships during the academic year 1945-1946, thirty during 1946-1947, fifty during 1947-1948 and sixty each academic year thereafter. Each scholarship consists of a cash award of \$600. Those eligible will include all students enrolled at universities to be selected by the RCA Education Committee. Selection of students will be made upon recommendation of the dean of the specified university and approval by the committee. The chairman of the Education Committee is Dr. James Rowland Angell, president emeritus of Yale University and public service counselor of the National Broadcasting Company.

A NEW list of all American Standards and War Standards approved to date has been published by the American Standards Association and is available free of charge. There are approximately eight hundred standards listed in the booklet, covering specifications for materials, methods of tests, dimensions, definitions of technical terms, procedures, etc., in the electrical, mechanical, building, transportation, textile and other fields. For ready reference, the standards are listed alphabetically as well as by engineering fields. There is also a separate list of war standards.

THE Beit Trustees have established a Beit research fellowship to be attached to the Rhodes Livingstone Institute for Social Anthropological Research in Southern Rhodesia. The value of the fellowship is £1,250 a year for four years.

It is reported in *Nature* that the London, Midland and Scottish Railway has announced a scheme whereby it will send members of its research staff for varying periods to carry out fundamental research in their particular fields in university laboratories; and, in exchange, the universities will be invited to send members of their staffs to spend a period in the company's research laboratory at Derby, working on applied problems in which they are interested from the fundamental side. The laboratory has a staff of seventy research workers, and has sections dealing

with engineering, metallurgy, chemistry, physics, paint and textiles.

THE Czechoslovak universities were closed by the German occupational authorities as early as November, 1939, and the libraries were depleted of many valuable volumes. In addition to this calamity, Czechoslovak scientists and research workers were deprived of the benefits of being informed about the American progress in science because they were cut off from American books and journals for a period of more than six years. There is a deep interest in Czechoslovakia in reprints of articles on antibiotics, enterobacteriaceae, sulfa drugs, the Rh factor, disinfectants, control of milk and water, Rickettsial diseases, chick embryo technique, fluorometric tests, vitamins, plant diseases, animal husbandry and poultry raising, food preservation, canning, etc. It would be very much appreciated if authors of any such articles would be kind enough to send some of their spare reprints to American Friends of Czechoslovak Institutions of Higher Learning, who would forward the collected copies to proper places as soon as shipping space becomes available. These should be addressed to L. Stolf, M.D., President, Czechoslovak Institutions of Higher Learning, 2401 Gunderson Ave., Berwyn, Ill.

It is reported in *The Times*, London, that "a four-fold indictment of German professors and scientists who condoned and even took part in the Nazi prostitution of learning was approved unanimously at New College, University of Oxford, on July 1, by the annual conference of the International Association of University Professors and Lecturers. The resolution condemned those who both before and during the war furthered the Nazi régime by propounding and spreading false biological theories and so making themselves morally responsible for racial persecution. Contributed, actively or passively, to the prostitution of science for the purposes of total warfare. Were active in the destruction of university libraries, scientific laboratories and museums in the German-occupied countries. Made use of modern developments in science, particularly medicine and physiology, in order to perform, on prisoners and civilians in concentration camps, experiments resulting in mutilation or death."

SPECIAL ARTICLES

TROMBICULA FLETCHERI WOMERSLEY AND HEASLIP 1943, A VECTOR OF TSU- TSUGAMUSHI DISEASE (SCRUB TYPHUS) IN NEW GUINEA¹

VERY early in the war with Japan, the occurrence

¹ From the United States of America Typhus Commis-

of tsutsugamushi disease (scrub typhus, mite fever) became a matter of military importance to troops in

sion, War Department and the Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army, Washington, D. C.

New Guinea. As a result of a request from the Commanding General, Southwest Pacific Area, to the Surgeon General of the Army, a commission was formed under the auspices of the United States of America Typhus Commission and the Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army to investigate the disease in this area. A complete account of these investigations is in process of publication.² A brief report is herewith presented of the findings with regard to a species of mite apparently responsible for the transmission of the causative agent, *Rickettsia orientalis*, in New Guinea.

The work of Japanese investigators from 1899 to 1931 established *Trombicula akamushi* (Brumpt) 1910 as the principal, if not only, vector of the disease on Honshu, Formosa and the Pescadores Islands. A closely related species, *T. deliensis* Walch 1923, has been found to be the most common trombiculid mite parasitic in the larval stage upon wild rodents and attacking man in the endemic foci of Sumatra,^{3,4,5} the Federated Malay States,^{6,7,8} India⁹ and North Queensland.^{10,11} Up to the present time investigators have failed to bring experimental proof of its role as a vector by recovering *R. orientalis* from susceptible animals which had been injected with emulsions of "ground-up" mites or from animals on which mites had been allowed to feed, although a highly suggestive result was obtained in one experiment with a gibbon.⁵

In New Guinea, observations on mites in relation to "endemic typhus" were begun by Gunther¹² before the war. The evidence at his disposal led him to suspect *T. buloloensis* Gunther 1939 as the most likely vector, but he had been unable to test this conclusion, at the time of his report, by injection of an emulsion of suspected mites into white mice. A mite which Gunther, in 1940, classified under the name *T. vanderghinstei* (subsequently identified as *T. deliensis* by Womersley and Heaslip), obtained from rats at Bulolo, New Guinea, was dismissed from consideration because of limited distribution.

The investigations of the commission were made in an area on the northeast coast of New Guinea which is triangular in shape and delimited roughly by lines connecting Oro Bay and Buna with a native village called Inonda, which is on the Samboga River approximately 20 miles inland. This locality, known as the Dobadura area, was one of the advanced bases of supply for the military operations conducted during 1943 and 1944. A clinical and epidemiological investigation of the occurrence of scrub typhus or tsutsugamushi disease among the Army units moving into and through this base was made during the fall of 1943.

In attempting to identify the species of mite responsible for transmission in this area, larval forms¹³ were collected not only from localities where cases were known to have originated and where as a result of use of the camp site extensive environmental changes had taken place, but also in similar places nearby, where natural conditions had remained undisturbed.

According to the method of collection, the larvae were divided into three groups:

- (1) Those found attached to the skin of individuals who had been exposed in the field;
- (2) Those which were attracted to and climbed upon the boots of the observer standing in an infested spot—"boot-collections";
- (3) Those which were found attached to the skin of small animals captured in the endemic area.

Collections of mites which had become attached to the skin of individuals were few in number. Exposure of volunteers for the purpose appeared to be injudicious in view of the risk of infection with scrub typhus. The species identified from this source of material were *Schöngastia blestowei* Gunther 1939, *S. pusilla* Womersley 1944 and *T. buloloensis* Gunther 1939.

"Boot collections" were made in a large number of localities associated with the occurrence of cases of tsutsugamushi disease. The same three species mentioned above were found most frequently, and a

² F. G. Blake, K. F. Maxcy, J. F. Sadusk, Jr., G. M. Kohls and E. J. Bell, *Am. Jour. Hygiene*, in press.

³ E. W. Walch, *Kitasato Arch. Exp. Med.*, 5: 63, 1923.

⁴ *Idem*, *Kitasato Arch. Exp. Med.*, 6: 235, 1925.

⁵ E. W. Walch and N. C. Keukenschrijver, *Archiv für Schiffs- und Tropen-Hygiene Pathologie und Therapie Exotischer Krankheiten*, Beiheft 1, 29: 420, 1925.

⁶ B. A. R. Gater, *Trans. of the Eighth Congress, Far Eastern Assoc. of Trop. Med., Siam*, 2: 132, 1930.

⁷ *Idem*, *Parasitol.*, 24: 143, 1932.

⁸ R. Lewthwaite and S. R. Savor, *Lancet*, 1: 255 (Part I), 1940; *Lancet*, 1: 305 (Part II), 1940.

⁹ D. R. Mehta, *Ind. Jour. Med. Research*, 25: 353, 1937.

¹⁰ W. G. Heaslip, *Med. Jour. Australia*, 2: 555, 1940.

¹¹ *Idem*, *Med. Jour. Australia*, 1: 380, 1941.

¹² Carl E. M. Gunther, *Proc. Sixth Pacific Science Congress of the Pacific Science Assn.*, 5: 715, 1942.

¹³ Specimens were identified quite largely on the basis of the papers "The Trombiculinae (Acarina) or Itch-mites of the Austro-Malayan and Oriental Regions," by H. Womersley and W. G. Heaslip (*Trans. Royal Soc. S. Australia*, 67: 68, 1943), and "Notes on and Additions to the Trombiculinae and Leewenhoekiinae (Acarina) of Australia and New Guinea," by H. Womersley (*Trans. Royal Soc. S. Australia*, 68: 82, 1944). In these two papers 33 species are recorded for New Guinea, a few of which may be regarded as of tentative rather than of established validity and are still under consideration by Womersley and others. This applies to the species *Trombicula fletcheri* and *Trombicula walchi*, to which reference will be made later. We are indebted to Mr. Womersley, South Australian Museum, Adelaide, for checking identifications of representative specimens collected in our investigation.

fourth, *T. kohlsi* Womersley 1944, was common. Two species less commonly found are particularly important, namely, *T. fletcheri* Womersley and Heaslip 1943 and *T. walchi* Womersley and Heaslip 1943. Four species were rarely found in boot collections: *T. scincoides*, Womersley 1944, *Guntherana bipygalis* (Gunther) 1939, *Guntherana parana*, Womersley 1944 and *Leeuwenhoekia nova-guinea*, Womersley 1944.

From boot collections and from the previous observations of McCulloch¹⁴ it was apparent that *S. blestowei*, *S. pusilla* and *T. buloloensis* were abundant and widely distributed. These three species appeared to be principally responsible for the "scrub itch" from which troops suffered in certain areas, but it seemed unlikely that they served as vectors of *Rickettsia orientalis*. They continued to be present in considerable numbers in infected localities after the incidence of tsutsugamushi disease had become sporadic or ceased altogether. There was no correlation between the occurrence of "scrub itch" and that of tsutsugamushi disease. Patients with this disease gave no history of itching at the site of the primary lesion or eschar produced by the infective feeding. These lesions or eschars were found on various parts of the body, while the lesions of "scrub itch" were characteristically distributed on the ankles, legs and, to a lesser extent, over the lower trunk.

The survey of the mites infesting local host animals in the Dobadura area of New Guinea involved the examination of 55 rats,¹⁵ 28 bandicoots (*Echymipera cockerelli*), 1 wallaby, 2 birds and 3 lizards. On small animal hosts larvae were found attached singly at various sites or in closely packed orange-red or whitish colonies numbering up to 300 or more individuals in each. Colonies were composed of one or more species and were usually situated in the conchae of the ears, on the ear margins and in the region of the genitalia.

In view of the previous work with tsutsugamushi disease, attention was concentrated upon rats and a marsupial, the bandicoot, since the possibility existed that the reservoir host of *R. orientalis* would be found among these animals. No rodents other than rats were found in the area concerned. Interest in mites was centered upon larvae of the genus *Trombicula*, although 5 other genera were represented in the collections from animal hosts. Table 1 summarizes the identifications of mites of this genus from rats and bandicoots.

¹⁴ R. N. McCulloch, *Med. Jour. Australia*, 2: 543, 1944.

¹⁵ Species determinations were made through the courtesy of Mr. E. L. Troughton, of the Australian Museum, Sydney, as follows: 29 *Rattus concolor browni*, 7 *R. mordax*, 3 *R. praetor* (?), 5 *R. gestri* (?), 7 *Melomys* sp., 1 *Uromys lamington*, 3 rats, sp. undetermined.

TABLE 1

Mite species	Found on rats		Found on bandicoots	
	Number of animals	Number of specimens	Number of animals	Number of specimens
<i>Trombicula fletcheri</i> .	25	250	22	399
<i>Trombicula deliensis</i> .	3	40	0	0
<i>Trombicula walchi</i> .	4	5	5	28
<i>Trombicula buloloensis</i>	2	2	3	4

T. fletcheri, a species closely allied to *T. akamushi*, was by far the most prevalent and abundant species on both rats and bandicoots. Since it had been encountered in boot-collections it presumably also attacked man. Next in importance on these hosts was *T. deliensis* and the closely related if not identical species, *T. walchi*, which had also been taken in boot-collections. *T. buloloensis* was least frequently taken. Few *S. blestowei* and no *S. pusilla* were found on these hosts.

In an effort to recover *R. orientalis*, eight pools of larval mites (7 from bandicoots and 1 from a rat, *R. mordax*) were emulsified and injected intraperitoneally into groups of 4 mice, each group receiving the emulsion from a single animal. Part of each pool was retained for species identification. The results with six of these pools were negative; two were positive. Both were from bandicoots.

From one (Host 21) over 300 larval mites were removed. Approximately 100 were retained for identification and the rest used for the inoculation. All the 69 specimens which were examined were classified as *Trombicula fletcheri*. From the other bandicoot (Host 22), similarly infested, about 90 mites were retained for identification and the rest, about 150 or more, were injected into mice. In the sample lot which was examined there were 63 *T. fletcheri*, 1 *T. walchi* and 1 *Guntherana parana*.

The rickettsial strains which originated from these two mite pools were designated No. 21 mite strain and No. 22 mite strain. These strains were continued by passage in mice and compared with strains recovered by inoculations of mice with blood from patients suffering from tsutsugamushi disease infected in the same area. Immunity tests between the strains of mite origin and a strain of human origin carried out in rabbits, hamsters and Swiss mice indicated complete cross protection. The human and mite strains were identical in behavior and exhibited the characteristics of *R. orientalis*.

Brain tissue from both bandicoots (Hosts 21 and 22), injected into mice, failed to produce rickettsial infection. Up to the present there is no convincing evidence that the bandicoot is a reservoir host. Since *R. orientalis* may be passed in mites from one gen-

eration to the next, it does not follow that the positive pools were infected by feeding on Hosts 21 and 22. The infection may have originated in the larvae of a previous generation feeding on another host species.

The evidence presented above establishes the recovery of *R. orientalis* from *Trombicula fletcheri* and indicates that this species of trombiculid mite serves as a vector of tsutsugamushi disease in northeast New Guinea.

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AGGLUTINATION OF STAPHYLOCOCCUS AUREUS IN THE PRESENCE OF CHORIO-ALLANTOIC FLUID FROM HEN'S EGGS^{1, 2}

CERTAIN strains of *Staphylococcus aureus* will agglutinate when grown in the presence of normal³ or influenza (PR8) virus-infected chorio-allantoic fluid. However, egg fluids vary in their ability to manifest this phenomenon, as is shown by the fact that a clumping of bacteria will occur in a dilution of 1 to 64 with some fluids and only 1 to 4 with others. Moreover, various strains of staphylococci, when cultured in the same chorio-allantoic fluid, may differ in their ability to show agglutination. Therefore it appears that there are two principal variables involved in the manifestation of this reaction, one associated with the organism, determining whether it will or will not be refractory, the other present in the fluid, determining whether or not it will exhibit the effect.

The phenomenon may be demonstrated by adding a drop of a six- to eight-hour nutrient broth culture of susceptible *Staphylococcus aureus* to varying dilutions, in extract broth, of normal or virus-infected chorio-allantoic fluid. These cultures are then incubated over-night at 37° C. and observed the following morning after being held at room temperature for an hour. Readings are made on a rough quantitative scale in a manner similar to that used in the conventional agglutination test; assigning values ranging from 1+ to 4+.

A wide variety of bacteria, including Gram-positive

¹ Preliminary studies.

² This investigation is aided by grants from the Fluid Research Funds, Yale University School of Medicine, and the International Cancer Research Foundation.

³ The term "normal" as used in this paper with reference to chorio-allantoic fluid and mouse lung filtrates is in contrast to the influenza-infected materials. It does not imply that these "normal" substances may not possess other anomalous agents which may support this agglutination phenomenon.

and negative cocci and rods, have been tested, but of those investigated only certain strains of *Staphylococcus aureus* and possibly *Staphylococcus albus* definitely participate in the reaction. *Staphylococcus citreus*, tested a few times, has been consistently refractory. The phenomenon may be elicited by using heat-killed staphylococci or living bacteria. While the agglutination takes place in a higher dilution of egg fluid when killed organisms are used, the clumps formed are usually smaller and the extent of reaction from tube to tube may vary irregularly. All the present observations were made with living bacteria.

Of the 31 cultures of *Staphylococcus aureus* studied, including 26 freshly isolated strains from human sources, two of animal origin and three laboratory strains, 10 of the human pathogens and one of the laboratory cultures were clumped when grown in egg fluids. While most of the agglutinable strains give an occasional negative reaction, those bacteria which are refractory are consistently so. The most outstanding example of the latter is the Wood 46 strain. On the other hand, a freshly isolated strain of staphylococcus, designated as "Craig," has always agglutinated in the presence of egg fluids infected with influenza virus and practically always with normal fluids. These two strains, therefore, have been used to evaluate the relative potencies of various chorio-allantoic fluids. Of 106 tests run with the Craig strain against normal fluid, only 11 failed to yield clumping. Of the 48 tests carried out with Craig and the virus-infected fluids, all showed agglutination. The Wood 46 strain has never shown agglutination with either normal (43 tests) or virus-infected (81 tests) egg fluids.

The agglutination of susceptible bacteria by chorio-allantoic fluid is not limited to any one medium in which the organisms may grow. All the media tried—extract broth, infusion broth, "Savita" and peptone broth—support the phenomenon. Everything else being equal, the extent of agglutination in these various media appears to depend upon the degree of growth or the number of the organisms present. However, there is one exception to this generalization, in that for some reason yet to be determined, susceptible staphylococci growing in a medium of vitamin-free casein digest, nicotinic acid, thiamin, biotin and salts are completely refractory to the effect of all chorio-allantoic fluids, normal and virus-infected.

It does not matter so far as the qualitative aspects of the test are concerned whether the staphylococcus-egg-fluid mixtures are incubated at 37° C. or at room temperature. If the growth is too heavy, the test may prove difficult to interpret. Most of the tests have been run at an original pH of 7.3, which may be reduced to around 6.8 after 18 hours' incubation. However, further studies have shown that the reaction