Meetings

Rickettsiae and Rickettsial Diseases

Advances in chemotherapy and public health and the decline in incidence of infectious diseases are partially responsible for the relative neglect in recent years of some bacterial pathogens, among them, the rickettsiae, the etiologic agents of epidemic and murine typhus, scrub typhus, the spotted fevers, Q fever, and trench fever. One or more of these diseases are still endemic in most parts of the world. Louse-borne epidemic typhus through its capacity to cause massive outbreaks of debilitating and often fatal disease, has dramatically influenced history. Although the danger of typhus has lessened with modern means of control, the disease persists in several areas of the world, and good judgment dictates that research on these medically important and biologically puzzling microorganisms should be continued. An international symposium in this field provides welcome stimulation.

The Czechoslovak Academy of Sciences, under its unique system of science planning [*Science* **154**, 924–926 (1966)] supports a broad and imaginative program of research on rickettsiae. It is not surprising, therefore, that a symposium was organized in Czechoslovakia by academician D. Blaškovič of the Institute of Virology, with the cooperation of R. Brezina and N. Kordová of the Department of Rickettsiology. The meeting was held 26–29 September 1967 in the castle of Smolenice near Bratislava. The participants included approximately 64 scientists from 15 countries.

Rickettsiae, with one notable exception, have not been grown on cell-free media and in the past they were considered to be intermediates between bacteria and viruses. It is now recognized that no form of life can be properly classified as intermediate between a bacterium and a virus and the rickettsiae definitely belong to the bacteria. The anatomy of the rickettsial cell has been studied by several investigators. At this meeting the bacterial nature of the principal cell constituents (cell wall, cell membrane, nucleic acids) of several species was carefully documented (Higashi, Bird, Schramek). One of the agents, *R. sennetsu*, suspected to be the cause of infectious mononucleosis in Japan, was shown to be somewhat different morphologically from the other rickettsiae and its taxonomic position in the genus *Rickettsia* was questioned (Higashi).

In addition to multiplication by binary fission, do rickettsiae have a developmental cycle that is not typically bacterial? This question was discussed in some detail. In the genus *Chlamydia*, the host cell is initially infected by a small compact form with an electron dense center which develops into a large reticulate body prior to multiplication by cell division. Small and large forms were described also for the agent of Q fever, *Coxiella burnetii* (Nermut), but were not seen in other rickettsiae. Small and large forms may correspond to spore-like and vegetative forms and need not represent a departure from events seen in bacteria (Armstrong). Physiologically, they may constitute repressed and derepressed cells (Weiss). The results of one-step growth experiments of *C. burnetii* in L cells suggested the existence of an initial eclipse phase (Kordová).

Biochemically, there are many similarities between rickettsiae and other bacteria. Numerous enzymatic activities involving several systems have been demonstrated. Essential enzymes may be missing or may remain inoperative outside a host cell, but what the deficiencies are and why rickettsiae fail to grow on cell-free media remain unknown. The only cultivable rickettsia is *R. quintana*, the agent of trench fever. Although this microorganism has a metabolism superficially resembling that of the other rickettsiae, the composition of the blood agar medium supporting its growth has not yielded clues as to what is needed by the other rickettsiae (Paretsky, Weiss, Wisseman).

Although cell culture techniques have not contributed to the improvement of methods of isolation and identification of rickettsiae, they have been of great value in studies of host-parasite interrelations. Kokorin presented phase contrast cinemaphotomicroscopic evidence that *R. siberica* is capable of active independent movement in the cytoplasm of infected cells. Motility was also observed in *R. prowazekii* and *R. conorii*, although none of these rickettsiae has recognized organelles of locomotion. Another interesting development is the growth of several rickettsiae in tick tissue culture. Reháček observed distinctive morphological differences in the appearance of the various species proliferating in primary explants derived from *Hyalomma dromedarii*. Comparative titrations with *R. conorii* and *R. akari* revealed that tick cell cultures were more sensitive than chick embryos. Tissue cultures were also used for detailed and quantitative observations of previously described phenomena. Surdan used inhibition of plaque formation as the method of study of the effect of oxytetracycline on rickettsiae. Pospíšil was able to isolate phase I *C. burnetii* following 11 passages of phase II in a continuous line of kidney cells of the monkey. Kazár reported that initial infection of chick embryo cell cultures with *C. burnetii* interfered with the subsequent growth of certain viruses. Sindbis, western equine encephalomyelitis, and vesicular stomatitis viruses were the most markedly inhibited; pseudorabies and vaccinia to a lesser extent; and Newcastle disease virus proliferation was not affected. Although interferon was produced by L cells infected with Q fever after 6 to 8 days, it could not be detected earlier when viral inhibition was present.

Research more directly associated with possible methods of prevention of rickettsial diseases occupied about half of the meeting. One of the difficulties encountered in the preparation of *C. burnetii* vaccines is phase variation, an alteration similar to the smooth-rough changes observed with certain gram-negative bacteria (Fiset). Although considerable progress has been made in the physicochemical separation of antigens from the two phases, even the purest phase I preparations contain phase II antigen (Brezina). The natural disease is produced by the phase I organism and experimental phase I vaccines provide better protection than phase II vaccines (Brezina, Fiset). Immunity in guinea pigs is reflected by an increased ability of peritoneal monocytes to phagocytize cells of phases I and II (Downs). An allergic response with formation of sterile abscesses is a com-

plication occasionally encountered with *C. burnetii* vaccines. It was shown by Ormsbee that in guinea pigs immunized with either *C. burnetii* or *R. prowazekii*, agglutinins and complement fixing antibodies are localized in $7S_{\gamma 2}$ serum fraction, while antibodies causing passive cutaneous anaphylaxis were found in the $7S_{\gamma 1}$ fraction.

Live vaccines against Q fever and epidemic typhus fever have been tested on a large scale in human volunteers in the Soviet Union. Currently, a typhus vaccine which is a combination of attenuated living and killed organisms is favored although a recent vaccine prepared from a fraction of typhus soluble antigen shows considerable promise (Zdrodowsky, Golinevich). A new Q fever vaccine which is given orally evoked serologic conversions in a majority of human volunteers in recent field tests (Genig). Typhus vaccine booster responses in individuals previously sensitized 5 years earlier by either natural infection or primary vaccination are indistinguishable. The importance of determining the complement fixation titer of the Cox-type killed vaccine in order to recognize potent vaccines was emphasized (Murray).

Newly described or improved serologic procedures include a passive hemagglutination test in which cysteine is used to differentiate 19*S* and 7*S* typhus antibodies (Voronova), and a rapid hemolysis-inhibition test for neutralizing antibodies in human serums (Mikolajczyk). Studies with *R. quintana* grown in blood agar indicate that suspensions of the whole organism serve as complement-fixing antigens and give no cross-reactions with convalescent serums from other rickettsial diseases (Vinson).

Successful vaccines for *R. tsutsugamushi* have not yet been achieved, mainly because several distinctive serologic types occur in nature and sufficient antigenic mass is difficult to obtain. Group- and type-specific antigens have been prepared from various strains of *R. tsutsugamushi* grown in tissue culture (Shishido). Prophylactic immunization of mice against homologous and heterologous strains was accomplished with chemo-vaccines composed of a mixture of live rickettsiae and a rickettsiostatic amount of tetracycline (Kekcheeva).

The involvement of domestic animals and ticks in an extrahuman cycle of *R. prowazekii*, originally described by Reiss-Gutfreund, stimulated several investigators to try, not yet successfully, to confirm this finding and to evaluate what might be a potentially serious public health problem. Gaon reported that family outbreaks of epidemic typhus in Bosnia were associated with a prior occurrence of Brill-Zinsser disease, and that cattle, sheep, and *Dermacentor marginatum* and *Ixodes ricinus* ticks infesting them were not infected. Lambs, kids, camels, and donkeys, experimentally infected with *R. prowazekii* in Egypt by Ormsbee developed typhus agglutinins, but rickettsemia could not be demonstrated and ticks fed upon the animals were not infected. Burgdorfer showed that the ZRS strain of *R. prowazekii*, originally recovered from *Hyalomma truncatum* in Ethiopia, when inoculated intracoelomically, would not propagate in female *H. excavatum* and killed the majority of *D. andersoni*. *H. dromedarii* sustained the rickettsiae but transovarial transmission did not occur. Thus, the accumulating evidence suggests that involvement of livestock and ticks, if it does occur, is secondary to active dissemination of the louse-borne disease in the human population and the extrahuman cycle does not play an important role in perpetuating endemic foci of the disease.

The importance of maintaining constant surveillance for rickettsial diseases was exemplified by reports of previously unrecognized epidemiologic and ecologic features. Strains of *R. tsutsugamushi* recovered from small rodents and insectivores, as well as from three species of *Leptotrombidium* and two species of *Neotrombicula* chiggers, collected in foci of scrub typhus recently recognized in the Khasan district of the Primorie (north of Vladivostok) and in the Pjandge and Sorbo river valleys in Tadzikistan in the U.S.S.R., were described by Tarasevitch. In regions of Thailand where at least five, and possibly eight, antigenically distinctive strains of *R. tsutsugamushi* were enzootic, Elisberg found the majority of scrub typhus patients were infected coincidentally with mixtures of antigenic types.

New enzootic foci of Q fever in Croatia, Yugoslavia, were attributed by Hrabar to the introduction of flocks of infected sheep and goats traveling from neighboring Bosnia and Herzegovina. Reptiles were implicated as possible reservoirs of Q fever. *Coxiella burnetii* persisted in the internal organs of lizards and water snakes following experimental infection and *I. ricinus* were

infected after feeding on the lizards (Ač). Zhmayeva reported that three species of Ixodidae ticks could be simultaneously infected with *C. burnetii* and tick-borne encephalitis virus after feeding. Both agents were transferred transstadially and transovarially.

Marked antigenic heterogeneity was found among various strains of the etiologic agent of bovine tick-borne fever, recovered in the same and in different geographic regions of Finland (Tuomi). A new group of rickettsia-like agents recovered from laboratory guinea pigs was described by Bozeman.

The review papers presented at this meeting will be published in *Zentralblatt für Bakteriologie* (Referate); those dealing with ecological and epidemiological problems in *Journal of Hygiene, Epidemiology and Microbiology* (Prague); and the others in *Acta Virologica.* The success of a symposium of this nature can be judged by the breadth of the interests which it spanned and stimulated, from problems directly related to human welfare to basic aspects of rickettsiae as distinctive biological entities.

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Calendar of Events

National Meetings

February

11–12. Congress on **Medical Education,** 64th annual, Chicago, Ill. (C. H. William Ruhe, AMA Council on Medical Education, 535 N. Dearborn, Chicago 60610)

12–14. **Aircraft Design** for 1980, Washington, D.C. (Meetings Manager, 1290 Sixth Ave., New York 10019)