

THE PROBLEMS OF THE PROTECTION OF MAN AGAINST TYPHUS

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Much has been said or published during the last few years about the possibility of epidemics of Typhus Fever due to prevailing conditions, such as occurred during and after World War I. Perhaps some have exaggerated the danger but even the most optimistic have not underestimated the destructive power of the disease. However, in spite of the advances made in the knowledge of the disease little has been added to the fundamental recommendations of Nicolle to reinforce our defenses against typhus.

The demonstration that rats harbor the virus and that fleas can transmit the disease from one rat to another, being infective for periods of at least 50 days, explained the numerous cases of typhus in men in the absence of the well accepted louse vector. The addition of fleas to the epidemiology of typhus was a rather serious complication in the problem of prophylaxis since a fight against rats is extremely difficult. On the other hand, the properties of the new type of typhus were to some extent different from those of the classic type but capable of producing identical infection in men and related to each other by a solid cross-immunization after infection.

With the discovery of rickettsiae in the tunica vaginalis of guinea-pigs infected with the orchitic typhus of Mexico, later found to be identical with the murine disease, Mooser awakened the hope that the virus could be cultivated in sufficient amounts for the production of vaccines. The work of Zinsser, Batchelder, Castañeda, Mooser, Varela and Parada contributed toward obtaining vaccines of sufficient antigenic content for human immunization. These authors worked with Mooser's virus while at the same time, Weigl in Europe perfected his method of production of a vaccine, cultivating classic virus in the intestinal tract of lice. The Zinsser-Castañeda vaccine proved to be highly effective against murine typhus but much less for classic typhus, while that of Weigl was far superior against this type of virus. The idea that the latter type of typhus is the important one in the production of epidemics has directed the efforts of most typhus workers to the production of classic vaccines (epidemic vaccines) giving little attention to what may happen in countries of high murine typhus incidence.

The usual mildness of Typhus Fever acquired directly from rats has, perhaps, been responsible for the secondary importance given to this

type of infection, in spite of the fact that rats are a constant source of virus capable of producing epidemics of high mortality rate, a condition observed in Mexico and undoubtedly also present in other countries where hygienic conditions are or may become similar. For this reason we consider it of interest to discuss the relationship of murine to classic typhus and also the convenience of a better understanding in regard to protection of men by means of vaccines.

I

The fact that murine typhus can be readily transferred from man to man when human lice are present should not be disregarded because: (a) it exists almost wherever rats live. Recently we found it in the tropics of Mexico (1) where it was not suspected before. It also was found in Cuba (2) and in Puerto Rico (3). (b) In the highlands of Mexico it may acquire a virulence that equals that of classic typhus. Therefore, it is reasonable to expect that epidemics of considerable importance are likely to appear whenever conditions such as those prevailing in war time would favor infestation by lice. We wish to mention some of the epidemics produced by a virus which most probably was murine typhus:

1917.—The epidemics of the Mexican-American border when Neil isolated the first known orchitic strains.

1928.—Typhus cases in Mexico City studied by Mooser (4).

1929.—Epidemic of Oaxaca, Mexico. Zozaya (5) without accepting their relationship with typhus, isolated orchitic strains from men and from lice.

1931.—An epidemic in Mexico City which it was claimed started from the old Belem prison. The virus was isolated for the first time from the brains of rats captured in that building (Mooser, Castañeda and Zinsser) (6).

1940.—An interesting study by Silva (7) on an outbreak of typhus in an asylum which killed two out of six patients. The virus was isolated from the patients and also from lice.

Most of the indicated data were directly known to the writer but more instances could be found not only of the epidemicity of murine typhus but also of its high virulence under epidemic conditions.

Even without the association of lice in the spread of the infection, murine typhus is becoming a rather serious problem. To have a general idea of its importance in the United States we may say that according to rough statistics there are more cases reported in this country than those of murine and classic typhus together in Mexico, a country which for many years has been one of the most afflicted with this plague.

The association of rats with the life cycle of typhus has stimulated interest in the improvement of measures for a better control of rodents and it is evident that well applied regulations for the destruction of such animals have given excellent results in well equipped communities, but results of campaigns in rural zones or in countries of little economic resources are far from satisfactory. Under such a state of affairs, the additional danger of epidemics due to the presence of lice, makes murine typhus a very complicated problem of never ending solution.

II

The discovery of murine typhus brought light to many unexplained lags in the epidemiology of the disease, but complicated the etiology to such extent that we have to accept "varieties of typhus" instead of the unitarian conception that previously prevailed. It is evident that a safe criterion for establishing the relationship between these varieties has been the consistent immunity to other strains shown by guinea-pigs infected with one strain. On the other hand, the experimental infection in laboratory animals shows differences which may be very conspicuous, mainly when typical murine and classic typhus are compared. Among such differences one of the most significant is that cross-immunization with killed rickettsiae is not complete or is even negative.

The early experiments on vaccination against typhus (8) showed that murine vaccines which had a considerable protective power against the homologous strains were active only in 1 out of 3 guinea-pigs tested against classic typhus. This was very striking because Weigl's vaccine made with classic virus was very effective against such type of typhus. Later we found that classic vaccine failed to protect against murine typhus in spite of its high potency when tested with the homologous strain. These findings were very discouraging for those who hoped to protect men by means of murine vaccines, mainly because they are more easy to prepare in comparison with the limitations in the cultivation of classic virus. To make the two types of vaccine more contrasting an incorrect terminology has been widely used in order to emphasize the epidemicity of classic typhus by calling it "epidemic" and suggesting the important role of murine typhus with the designation of "endemic."

The duality in the etiology of Typhus Fever was, therefore, a practical concept and each virus was placed in a very definite epidemiologic position. In regard to this question, we have discussed already the epidemiogenic capacity of murine typhus and therefore, need say little about a classification that besides being incorrect is rather misleading.

The apparent duality in the etiology of typhus is contradicted by the study of various strains isolated in Mexico. In 1930 (9) we reported on certain strains of typhus which showed properties of European virus and then, after a number of transfers acquired those of murine strains. Nicolle, who visited Mexico at that time, advised the term "intermedials" to designate such strains, suggesting that they were an indication of an intermediate stage in the transformation of murine to classic typhus. Later on, Mooser, Varela and Pils (10) transformed some classic-like strains to the murine type by means of certain laboratory procedures. More recently (11) a number of strains isolated in the same hospital in Mexico and during the same epidemic were very illustrative of a possible gradual transformation from murine to classic typhus. Typical murine and classic strains were found and also strains which acted first as classic to become spontaneously murine-like on subsequent transfers in guinea-pigs.

After such findings, we have considered it justified to assume that since all strains are fundamentally related, they must have a common and recent origin

and, that from this origin an evolution is constantly taking place resulting in a spectrum of variants until an extreme is reached where the acquired properties are very different from those of the original strain.

III

The finding of strains of typhus with properties of both murine and classic virus is a good argument in favor not only of a common origin but also of a constant evolution from one type to the others. However, this is a mere assumption that requires corroborative data before being accepted as a satisfactory hypothesis. These data can be obtained from a comparative study of the properties of murine and classic typhus and we think that the following summary of the important similitudes and differences between them, may be helpful in the understanding of this discussion.

The relationship between murine and classic strains has been based on:

- (a) Similar disease in man.
- (b) Similar serological findings.
- (c) In the presence of lice, similar epidemiology.
- (d) Cross-immunity after infection.
- (e) Morphology, intracellular position and other properties common to both types of rickettsiae.

The differences between murine and classic strains are:

- (a) Different epidemiology under ordinary circumstances.
- (b) Different pathogenicity for laboratory animals.
- (c) Different antigenic composition as demonstrated by complement fixation (12), neutralization tests and by cross-vaccination with killed rickettsiae.

The relationship based on the above indicated data is more consistent than that found among the different types of organisms such as pneumococci or meningococci. The typhus group, however, is far from being confused with the closely related Spotted Fever and Tsutsugamushi groups in spite of a certain immunologic relationship observed between Spotted Fever and typhus (12).

The differences which have been summarized are not fundamental and one may consider them as purely quantitative. As a matter of fact, the scrotal swelling in guinea-pigs which is the basis of experimental differentiation, can be found in animals infected with classic typhus and the infection which has the characteristics of being massive in rats infected with murine typhus can be detected in these rodents although to a minimal degree. The immunological differences, also can be overlapped by an adequate increase in the antigenic content of the vaccines or in the potency of the immune serum.

Because of its practical importance, the difference in antigenicity shown by cross-vaccination and neutralization tests deserves to be further discussed.

From the early experiments on vaccination we noticed that murine vaccines were not quite effective against classic typhus. Later (13) (14) it was demonstrated that, by increasing the amounts and doses of such vaccines, protection could be obtained against all known varieties of typhus. On the other hand, we made quantitative tests with vaccines prepared with classic strains and found that suspensions of rickettsiae which had a considerable protective power against the homologous virus failed to protect against murine typhus. These experiments, corroborated those of Veintemillas (15) who tested a lot of Cox's epidemic vaccine

against Tunisian murine strains. We also made experiments with neutralization of the virus by testing mixtures of antiserum with rickettsiae in the skin of guinea-pigs. It was found that the serum of guinea-pigs which had recovered from murine typhus inhibited the skin lesion produced by murine as well as classic virus, while, under similar conditions, classic immune serum protected only against the homologous strains. These various experiments have given us the impression that the murine type of rickettsiae have a range of protective activity considerably higher than that of the classic variety and that perhaps, the failure of the latter to compensate its deficiency by an increase in antigen, as is the case with murine vaccines, may be due to a considerable deviation from a more complete antigen such as the murine type.

Considering side by side the relationship and differences between murine and classic typhus as well as the interesting properties of the intermedial strains, it seems quite reasonable that in case of a full acceptance of a common origin for all typhus strains, the murine type is the representative of a normal strain which due to accidental transfer to a new host suffers changes in its pathogenicity and antigenic composition in a manner similar to what is constantly being observed in other species of the realm of micro-organisms. If conservative criticism does advise against the acceptance of a possibility of strain variation in rickettsiae strains we cannot but think of what happens with Rabies and Yellow Fever which give us indication of considerable variation shown by loss of pathogenicity to a host as a result of adaptation to a new one. The Typhus Fever classic strains seem to have lost their adaptability to rats which are the important natural reservoir of the typhus virus.

IV

The concept of strain variation in the typhus group helps to clarify epidemiologic and prophylactic problems in countries like Mexico where murine and classic virus produce epidemics of equal importance. The discussion which we have presented indicates that the protection of men by means of vaccines has to be considered after a careful analysis of the immunological properties of the prevailing strains at a certain time and in a certain zone. Therefore, it is not quite reasonable to decide that monovalent vaccines of any type would be convenient for all countries because they may be useful for some places. For Mexico and probably the rest of the Latin-American countries the use of the so-called "epidemic vaccines" is not recommendable because most of the strains causing epidemics, if not pure murine, are far from being all typical classic strains. Furthermore, the vaccines made with classic rickettsiae are very limited in their range of protection against heterologous strains. On the other hand, a murine vaccine of poor antigenic value is rather insufficient against most intermedial and classic strains. It is because of these considerations that we have advised the use of bivalent vaccines (16) hoping that they might cover all types of strains causing epidemics in Mexico. The protective value of the bivalent vaccine has been found to be very much superior to either murine or classic vaccines against both types of typhus. However, in spite of the high experi-

mental value of the combined vaccine we found that in order to establish efficacy for human protection one cannot rely on results of guinea-pigs tests. A lot of vaccine containing 4 times more antigen than that needed according to the potency tests was used for the vaccination of 200 persons with results which were far from being satisfactory. The experiment was undertaken by the Office of Epidemiology of Mexico and it was found that 10 vaccinated persons acquired typhus against 12 out of a control group of an equal number of individuals. The disease was milder in the vaccinated persons but the failure to fully protect indicates that the antigen has to be given in larger doses.

We wish to recall that experiments in vaccination of men have been made in Mexico (13) and in Bolivia (17) using as antigen vaccine of murine rickettsiae. It was observed that 3 or 4 doses of rather rich vaccines were sufficient to protect against amounts of murine and classic virus sufficient to infect guinea-pigs. The inoculum was administered by intradermal injection 14 to 30 days after the last dose of vaccine. Such experiments demonstrated that murine vaccines can protect against typhus provided they have sufficient antigen and that with such antigenic content men are also protected against classic typhus and very probably against all intermedial strains.

Subsequent experiments have given us more information about what may be expected from human vaccination. Groups of "contacts" numbering nearly 1,000 persons have been treated with monovalent or bivalent vaccines administering from 3 to 6 doses at weekly intervals. With about 800, one year has elapsed since the day of vaccination. Nearly 300 have been revaccinated and no cases of typhus have occurred among them. The remaining individuals are now being called for new treatment and we hope to hear from most of them. As contacts they are very much impressed by the cases that have occurred in their families and are usually willing to accept revaccination or to keep in contact with the laboratory.

The rather intensive treatment in "contacts" is showing contrast with the results from previous vaccines of weaker antigenic content. Several cases of typhus have been observed in nurses or students treated with 2 or 3 doses of moderate suspensions of rickettsiae and from various sources we have received information that about 30 out of 5,000 vaccinated persons developed Typhus Fever.

From the early attempts to protect men against typhus to the present time we have been handicapped by difficulties in producing sufficient amounts of rickettsiae in order to obtain vaccines of equal potency with those recommended by Weigl who succeeded in cultivating the classic virus in lice. The Zinsser-Castañeda vaccine prepared in rats contained large amounts of murine rickettsia but used in doses capable of protecting guinea-pigs against the homologous virus failed to protect against classic typhus. Such vaccines were improved by proper concentration of the antigen but in spite of the good yield of rickettsia cultivated by such method the production was far from being practical. The demonstration that classic vaccines were considerably more efficient against classic typhus encouraged research which resulted in the improvement of methods for the cultivation of this type of rickettsiae. Zinsser (18) recommended his agar media and Cox (19) developed to a practical method the use of developing chick embryos as a medium.

In 1938 (20) we found a practical method for the cultivation of murine rickettsiae by inoculation into the lungs of rodents, a method which was also successfully applied by Durand and Sparrow (21) to classic virus. The amounts of rickettsiae obtained are really astonishing and suspensions of any concentration and quite free from impurities may be prepared. Our suspensions of murine rickettsiae could, therefore, be arranged in order to overlap antigenic differences and be effective against all types of typhus. The association of classic rickettsiae cultivated in mice and murine rickettsiae cultivated in rats have in our hands given a practical method of producing bivalent vaccines of higher protective value than those formerly used.

We have made several attempts to produce classic vaccines in eggs according to Cox's methods but only small amounts have been obtained due to great difficulties in securing suitable material. The price of fertilized eggs is too high to be practical in Mexico and the yield in antigen far too inferior to that obtained from mice. A combined vaccine prepared from mice and rats is cheaper and the suspensions of rickettsiae can be concentrated to small volume to be diluted conveniently at the moment needed. The high rickettsial content compared with the minimal amount of impurities makes the danger of sensitization to foreign proteins very unlikely which, by the way, need not cause worry since such proteins have little chance of coming in contact with men.

The vaccines prepared according to the "lung method" and administered not only in large doses but in 5 or even 6 injections at weekly intervals have been giving very satisfactory results in preventing infection in persons exposed to air-borne infection during the process of preparation of typhus vaccine. The vaccinated persons may show a rather unpleasant reaction but never one as bothersome as that following typhoid vaccination. The results are in contrast with the facility with which non-vaccinated individuals acquire the infection when accidentally exposed to the virus.

One of the main reasons why the lung method is not recommended in some countries is the danger of infection of laboratory workers. The handling of infected material and the constant sneezing of the animals spreads a virus which has many ways of entering the organism. As we have indicated this danger could be reduced if a proper treatment were given before exposure to the virus.

SUMMARY AND CONCLUSIONS

A brief discussion has been presented on the etiology, epidemiology and immunology of Typhus Fever in relation to problems concerning the protection of man by means of vaccines.

It has been emphasized that epidemics of great severity can be observed with murine and intermediate strains as well as with classic typhus. Therefore, the

use of the terminology "epidemic" and "endemic" for classic and murine strains respectively, is criticized.

The immunological differences found between murine and classic strains are important but not fundamental and in that which concerns vaccination it has been demonstrated that murine vaccines can be useful against classic typhus provided a sufficient amount of antigen is given.

The properties of "intermediate" strains and the relationship and differences found between the two main types of typhus suggest that phenomena of strain variation are responsible for such differences and that murine typhus of wider antigenic range than classic strains is the representative of the "parent strain."¹

Human experiments have shown that "murine" vaccines, when administered in proper concentration are capable of conferring protection against murine and classic typhus. However, there is recommended for practical immunization the use of bivalent vaccines, hoping to improve the protective effect against all types of typhus.

In the Typhus Laboratory of Mexico "bivalent vaccines" are prepared in the lungs of mice and rats infected with classic and murine virus respectively. The vaccines are obtained in pure rickettsial suspensions and can be concentrated to any desired requirements.

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Las enfermeras y su misión.—Para ser enfermera se necesita en primer lugar tener vocación, saber que su vida va a hacer un constante sacrificio en beneficio de la salud colectiva. Su misión no es solamente atender al enfermo, debe aplicar los principios de higiene y profilaxia según el caso de los hogares donde se han solicitado sus servicios. Se necesita un alma bien templada y nervios de acero para cumplir con su deber, para no desmayar cuando todo a su alrededor es un lamento, cuando la carne enferma estalla en gritos de dolor y hay que acudir solícitos, afanosos a prestar los auxilios necesarios sin que el ánimo decaiga, sin dejar traslucir la compasión que nos inspira el estado del enfermo, para no amilanarlo, para que en vuestra calma y oportunos auxilios encuentre fuerza y energía suficientes para vencer el mal que lo aqueja.—LUIS A. ADRIANZA: *Ene*, agto.—sbre. 1943.