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THE TYPHUS GROUP OF FEVERS

CLASSIFICATION, LABORATORY DIAGNOSIS, PROPHYLACTIC INOCULATION, AND SPECIFIC SERUM TREATMENT*

BY

A. FELIX, D.Sc.

(From the Emergency Public Health Laboratory Service)

The "typhus group of fevers," comprising a number of different varieties of the disease, is now firmly established. Typhus researches during the past few years have revealed the occurrence of typhus-like diseases in many parts of the world. Not a few endemic diseases of various tropical and subtropical countries, some of which had long been known under the label "unclassified fevers," have now been recognized as members of the typhus fever group. This recent development is mainly due to the application of serological and immunological methods to the study of these diseases. The so-called Weil-Felix reaction has provided in almost every instance the first evidence of the generic relationship of the unknown disease, to classical louse-borne typhus, and thus opened the way for the experimental study which has led to the demonstration of the corresponding rickettsia. Some of the best-known instances of this sequence of events are: the endemic typhus of the United States of America (Maxcy and Havens, 1923); the tropical typhus of the Federated Malay States (Fletcher and Lesslar, 1925); the "fièvre exanthématique" of Marseilles, and "fièvre boutonneuse" of Tunis (Burnet and Olmer, 1927; Felix and Rhodes, 1931; Durand, 1932); São Paulo typhus (Piza *et al.*, 1931; Monteiro, 1931; Felix, 1933a); and the tick-bite fever of South Africa (Pijper and Dau, 1930, 1931, 1932).

The terms "rickettsial infections" and "typhus group of fevers" are not synonymous, the former being much wider than the latter. If the rickettsial infections that occur in certain domestic animals are excluded and only those that affect man are considered, there still remain some rickettsial diseases of man which, according to our present knowledge, seem to be unrelated to the typhus group of fevers. Among these diseases may be mentioned the "Q" fever of Queensland (Derrick, 1937; Burnet and Freeman, 1937) and of certain parts of the U.S.A. (Davis, Cox, Parker, and Deyer, 1938), and the "trench fever" which became known in 1916 as a wartime infection but has not again made its appearance in epidemic form, even during the present war.

Classification

Megaw (1921, 1930) was the first to attempt to classify the typhus-like fevers as members of one group of diseases and suggest a subdivision according to the transmitting insect vector. The suggestion was undoubtedly a step in the right direction, and so long as the group was composed of only a few diseases the differentiation according to vector seemed to fit the known facts. This, however, no longer holds good.

A satisfactory classification of the typhus group can be based solely on the antigenic type of the corresponding rickettsiae,

upon which immunity and immunity reactions depend. The antigenic analysis of the various typhus rickettsiae has, however, not yet passed its infancy. The most widely studied of these organisms is *R. prowazeki*, the causal organism of Old World and New World typhus of type X 19. It is known that *R. prowazeki* possesses a heat-labile and a heat-stable antigen (Castaneda and Zia, 1933), that the latter is common to *R. prowazeki* and *Proteus* OX 19 (Weil and Felix, 1921), and that this O antigenic component is alkali-stable (Bruce White, 1933). The antigenic structure of the rickettsiae of the other varieties of typhus and their relationship to each other have not been studied as yet, owing to the great difficulty in obtaining pure suspensions of rickettsiae in sufficiently large quantities. This difficulty has been overcome only quite recently, thanks to the discovery of two methods of growing rickettsiae—namely, in the yolk sac of the developing chick (Cox, 1938) and in the lungs of infected rats and mice (Castaneda, 1939). There is good reason for believing that as a result of these important advances the gaps in our knowledge will be filled very soon.

So far the serology of the typhus group has been based on the antigenic relationship between the rickettsiae of the different varieties of the disease and corresponding serological varieties of *Proteus* X. This relationship is most strikingly demonstrated by the close correlation between cross-immunity and agglutination reactions with the three types of *Proteus* X

TABLE I.—Provisional Classification of the Typhus Group of Fevers

Immunological Subgroup	Type OX 19	Type OX K	Type undetermined
Name of disease..	Classical epidemic typhus Tabardillo (Mexico) Brill's disease (U.S.A.) Endemic typhus of U.S.A. and Australia, Greece, Syria, Manchuria, Malay (shop typhus), India, Burma, Philippines, Hawaii, Toulon (fièvre nautique), etc.	Tsutsugamushi fever of Japan, Formosa, Malay, and Dutch East Indies Scrub typhus of Malay, Dutch East Indies, India, French Indo-China, Australia	Spotted fever of Rocky Mountains Spotted fever of eastern U.S.A. São Paulo typhus Fièvre boutonneuse (Mediterranean) Fièvre exanthématique of Marseilles Febbre errutiva (Italy) Tick-bite fever of South Africa Epidemic and endemic typhus of South Africa Tick-borne typhus of India, Kenya, etc.
Vector ..	Lice and rat fleas	Mites	Ticks, lice, and rat fleas
Reservoir of virus	Rats. Man	Field mice and rats	Rodents. Dogs. ? Ticks. Man
Agglutination ..	OX 19 +++ OX 2 + OX K -	OX 19 - OX 2 - OX K +++	OX 19 + OX 2 + OX K +

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strains, known as OX 19, OX 2, and OX K (Felix, 1933b). The conception of serological varieties of typhus, based on the work of Fletcher and Lesslar (1925) and formulated by Felix and Rhodes (1931), led to the subdivision of the typhus group into three immunological subgroups.

Table I shows that the suggested scheme (Felix, 1935) comprises at present only two well-defined main types—namely, types OX 19 and OX K. The third subgroup, provisionally designated “type undetermined,” probably contains several different types. Further subdivision will be made possible by the use of pure cultures of the corresponding rickettsiae, which will disclose the interrelationships of both the heat-stable and the heat-labile rickettsial antigens.

The most important of these diseases is the louse-borne typhus of type OX 19, known as the classical epidemic typhus and caused by *R. prowazeki*. The subsequent sections of this paper deal mainly with this variety of the disease, though some of the conclusions are applicable also to the other typhus-like fevers. A few remarks on nomenclature may not be out of place here. In the past some confusion has arisen from the tendency to use a number of different names to designate the same disease. In recent papers published in this country (Findlay, 1941a, 1941b) this tendency has reappeared, and the louse-borne epidemic typhus has been given a new name—viz., “exanthematic” typhus. It cannot be said that there was need of a new name. The one chosen by Findlay certainly does not offer any advantage over the generally accepted old names. There is nothing distinctive in the name “exanthematic” typhus, since all the varieties of typhus are exanthematous diseases. Louse-borne typhus occurs as an endemic disease in many parts of the world, but gives rise to widespread epidemics only under exceptional conditions, chiefly in the wake of war, famine, or other major disasters that favour louse-infestation. The term “louse-borne typhus,” used throughout this paper, applies to both the epidemic and the endemic form of the disease.

Laboratory Diagnosis

The clinical diagnosis of typhus is not altogether easy, so that early guidance is sought from laboratory findings. The following laboratory methods of diagnosing typhus infections have been suggested at one time or another: biopsy of skin lesions, with a view to demonstrating typhus nodules or rickettsiae; inoculation of the patient's blood into guinea-pigs or other laboratory animals; serological tests. The latter alone have proved to be of practical importance. It is, of course, theoretically possible to diagnose a typhus case by isolation of rickettsiae from the patient's blood or from the insect vector, when this has been found on the patient. It is, however, a mistake to recommend these methods for diagnostic purposes (Army Pathology Laboratory Service, Current Notes, 1941). These complicated and rather dangerous procedures would hardly ever enable the pathologist to aid the clinician in diagnosing a case of typhus fever.

Fortunately, the isolation of rickettsiae is not necessary, since the diagnosis of typhus can be confirmed by a relatively simple serological test. The Weil-Felix reaction has been employed for the purpose during the past 25 years. The limited space here available precludes discussion of the technique of the test, its application by the clinician and epidemiologist, its limitations, and the interpretation of results, but it can be stated that practical experience on a very large scale has shown that the *Proteus* OX 19 reaction is the most constant sign of louse-borne typhus; the clinical symptoms, on the other hand, may show very wide variations.

Experiments on agglutination of rickettsial suspensions have been carried out ever since Weigl (1923, 1930) first obtained fairly concentrated suspensions by his method of anal infection of lice, and Zinsser and Castaneda (1932) by intraperitoneal infection of x-rayed rats. These experiments, though of outstanding importance in typhus research, have not so far made it possible to adopt agglutination tests with rickettsiae as a routine method of diagnosing typhus cases. The microscopical technique gives unsatisfactory results, while the macroscopic technique requires relatively large quantities of rickettsial suspensions, which can be more usefully employed for the preparation of vaccines. The most recent advances in growing

rickettsiae (Cox, 1938; Castaneda, 1939) have not, for the time being at any rate, materially altered the position.

The published data on the agglutination of *R. prowazeki* by the serum of typhus patients and convalescents present a rather confusing picture. Some workers have reported almost complete parallelism between agglutination of *R. prowazeki* and *Proteus* OX 19 (Weigl, 1930; Zinsser and Castaneda, 1932), whereas others obtained quite different results (Liu, Zia, and Wang, 1938; Hudson, 1940). Obviously the confusion is due to the fact that agglutination of rickettsiae can be adequately described only in terms of two separate reactions—namely, those which occur between the heat-labile and the heat-stable antigens and their respective antibodies. According to Castaneda and Zia (1933), living or formalized rickettsiae are not agglutinated by the OX 19 antibody, but become agglutinable after heating at 75° C.; phenol seems to produce a similar effect to heat. Differently prepared rickettsial suspensions, such as have been employed by various workers, do not therefore indicate the same antigen-antibody reaction when tested against typhus sera that contain, or may contain, the two different antibodies.

What has been said about agglutination of rickettsiae applies also to complement-fixation with these organisms (Castaneda, 1936b; Mosing, 1938; Bengtson, 1941; Bengtson and Topping, 1941). Either of the two reactions may be used for the demonstration of the antibody corresponding to the heat-labile rickettsial antigen provided that a technique is devised by means of which the heat-stable O antigen is precluded from taking part in the reaction and thus obscuring the results.

Although the technique of the customary test-tube agglutination with *Proteus* OX 19 is very simple, still simpler procedures have been adopted by workers in the U.S.A. (Welch, 1937) and in Mexico (Castaneda *et al.*, 1940) in the form of slide agglutination tests. German workers even employ a slide test using dried drops of whole blood instead of the separated serum. Extensive surveys are being carried out by this method in endemic areas in occupied Poland, with a view to detecting missed ambulatory patients and cases of so-called inapparent infection. The latter cases are of special epidemiological importance since they do not show any clinical symptoms whatever, but are capable of infecting lice and spreading the disease. The dry-drop blood tests are carried out within a few minutes even under the most primitive conditions, and it is claimed that the results compare favourably with those obtained with test-tube agglutination (Eyer and Grützner, 1939-40; Steuer, 1942).

Immunity to Typhus in Relation to the Known Antigenic Constituents of Rickettsia

Immunity to typhus has been studied in experimental animals for the past 30 years, but is as yet very incompletely understood. The main obstacles to progress have been, and still are, lack of knowledge of the essential antigens of the causal organism and lack of a suitable experimental animal. Most of the work has been done on guinea-pigs and rabbits; more recently rats and mice have come into use. In none of these animals, however, does typhus infection run a course comparable to the disease in man. Only the chimpanzee reacts with symptoms similar to those of a mild case in the adult man. Monkeys of the various lower genera so far tested do not offer any advantage over the guinea-pig as test animal. Quite recently the Eastern cotton rat of the U.S.A. (Snyder and Anderson, 1942) and the common gerbil of South Africa (Gear and Davis, 1942) have been suggested as more susceptible animals, and are now being tried out.

It has been known for a long time that immunity to typhus is complex—that is to say, it is related to more than one antigenic constituent of the causal organism. The O antigen of *Proteus* X 19, which corresponds to the heat-stable antigen of *R. prowazeki*, does not induce immunity to experimental typhus infection, and the corresponding O antibody, though so valuable in diagnosis, does not account for the protective action of convalescent serum from man and animals. A clue to this puzzling problem has been provided by the work of Castaneda (1934, 1935, 1936a, b, c), who showed that *Proteus* OX 19 immune serum reacts with rickettsia only in agglutina-

tion, precipitation, and complement fixation, but is inactive in opsonization. It is the antibody to the heat-labile rickettsial antigen that is of outstanding importance in phagocytosis of *R. prowazeki* and in protection against infection with this organism.

It would be wrong, however, to conclude from these experiments that the heat-stable O antigen and its corresponding antibody are not concerned in typhus immunity, because a glance at Table I shows that they are. Cross-immunity between different varieties of typhus rickettsiae is accompanied by identity of the serum reactions to the O antigen of the corresponding *Proteus* X strains, whereas failure to obtain cross-protection coincides with dissimilarity in O agglutination reactions. The results of cross-immunity tests can thus be predicted from the O agglutination tests obtained with various types of *Proteus* X, and have in fact been so predicted (Felix, 1933b). It is also well established that there are two types of the curve of OX 19 agglutinin production in typhus patients, and that these are related to the clinical course of the disease (for references see Felix, 1930).

These facts can be reconciled with Castaneda's findings if it is assumed that the labile antigen of *R. prowazeki* plays a part similar to that of the labile somatic antigen of the typhoid bacillus, known as the Vi antigen. The analogy between the two is indeed very striking, for the typhoid O antigen alone is incapable of inducing in animals effective immunity to infection with virulent Vi + O bacilli, but the Vi antigen is. And, again, typhoid Vi antibody is a powerful opsonizing agent for Vi + O bacilli, but the O antibody is not. *R. prowazeki* and *Bact. typhosum* also show close similarity in the agglutinative properties of their somatic antigens. Living and formalized suspensions of both organisms are not agglutinated by their respective O antibodies but become agglutinable after heating. Breinl (1924) showed that the O antigen of *R. prowazeki* from the louse gut retains its agglutinogenic property after heating at 100° C., as do O antigens generally.

It seems, therefore, quite natural to assume that the O antigen of *R. prowazeki* plays a part similar to that of the O antigen of the typhoid bacillus and other *Salmonellas*, and of other organisms—namely, that of acting as an endotoxin. The corresponding OX 19 antibody would thus function as an anti-endotoxin (Boivin and Mesrobian, 1933; Raistrick and Topley, 1934; Felix and Pitt, 1934). Strong evidence in support of this view is provided by the clear-cut results of intradermal tests with heat-killed suspensions of *Proteus* OX 19 (Friedberger and van der Reis, 1919) or with extracts prepared from *Proteus* OX 19 (Fleck, 1931). In normal persons and non-typhus patients the intradermal injection of OX 19 antigen produces a local inflammatory reaction, reaching its maximum after 24 to 36 hours. Typhus patients react in a similar manner during the first few days of illness, but from the fifth or sixth day onwards the reaction is negative and remains so throughout convalescence and for varying periods, up to several months, after recovery. This period coincides with the presence in the serum of OX 19 agglutinins in significant titre. The toxicity to man of the OX 19 antigen and the specific neutralization of its toxic action as a result of recovery from typhus infection are thus clearly demonstrated.

The latest development in the study of typhus immunity is the demonstration by German workers of a heat-labile rickettsial endotoxin which is lethal to mice and specifically neutralized by convalescent serum from man and animals (Gildemeister and Haagen, 1940; Otto and Bickhardt, 1941). The lability of this toxic substance is much the same as that of the heat-labile rickettsial antigen described by Castaneda, and it seems very likely that the two are identical.

It would appear, therefore, that in two widely separated organisms, such as the typhoid bacillus and the strictly intracellular virus-like *R. prowazeki*, the mechanisms of infection and immunity are in principle very much alike. In both cases a heat-labile somatic antigen, besides acting as a toxic substance, inhibits the interaction between the O antigen and the O antibody, and thus protects the bacterial, or rickettsial, cell against the opsonizing and bactericidal actions of the natural and immune O antibody. It is not suggested that these are the sole factors associated with immunity to typhus. Antibodies

to yet other, still unknown, constituents of the rickettsial cell may also play a part. But it is obvious that the two rickettsial antigens and antibodies that have so far been defined are essential elements in immunity to typhus.

Specific Serum Treatment

Specific serum treatment of typhus fever of recognized value has not yet been evolved, and attempts at chemotherapy so far have also been unsuccessful. One of the German typhus experts working in Warsaw since 1940 recently reported that trials with nearly 100 different drugs, including 24 sulphonamide compounds, gave only negative results (Wohlrab, 1942). French workers who have had many years' experience of louse-borne typhus in Tunis consider treatment with sulphapyridine not only useless but even harmful (Durand and Balozet, 1941).

Convincing therapeutic effects have not been observed with convalescent serum, although such sera usually exert a definite prophylactic action when tested on the guinea-pig. Wohlrab (1942) found pooled convalescent serum ineffective not only in the treatment of typhus cases but even as a prophylactic when employed during a prison epidemic in Poland.

Durand and Balozet (1941) treated a series of 120 cases of louse-borne typhus in Tunis with serum from horses immunized with living rickettsiae from infected mouse lung and believed that they obtained evidence of its beneficial effects. It is, however, rather disappointing to note from their paper that only the indigenous patients seemed to respond to the treatment, while the European patients did not. Wyckoff and Bohnel (1942), using a strongly concentrated serum from immunized rabbits, described therapeutic effects in guinea-pigs infected with louse-borne typhus. The data published by these workers do not, however, warrant their conclusion, since the serum was in each instance administered before the onset of fever. The present position, therefore, is that there is no convincing evidence of the curative action of the so-called therapeutic sera, although their prophylactic value in animal experiments has been established beyond doubt.

In the light of the facts discussed in the preceding section it would appear that the efficacy of a therapeutic anti-typhus serum depends upon the presence in it of effective amounts of the two different antibodies corresponding to the heat-labile and heat-stable rickettsial antigens. It is, however, quite clear that sera of this kind have not yet been employed in the treatment of typhus patients. Convalescent sera cannot be obtained sooner than two or three weeks after defervescence, by which time the OX 19 titre has fallen to a mere fraction of its maximum value. In louse-borne typhus this maximum is usually reached a day or two before or after the first day of defervescence. There can be little doubt also as to the deficiency in O antibody content of the therapeutic immune sera so far prepared. Durand and Balozet (1940) showed that prolonged hyperimmunization of horses and donkeys in no instance resulted in an increase in the low-titre OX 19 antibody which each of the animals had developed at the very beginning of the immunization. While the titre of the neutralizing antibody rose that of the OX 19 antibody fell, although the doses injected contained enormous numbers of rickettsiae.

TABLE II.—Showing OX 19 Agglutinin Titres of Therapeutic Anti-typhus Sera and Sera from Horses Immunized with *Proteus* OX 19 Antigen

Serum	Antigen used for Immunization	OX 19 Agglutinin Titre
Anti-typhus serum (horse) (Durand and Balozet, 1940)	<i>R. prowazeki</i> from mouse lung. Tunisian louse-borne strain	1:100
Anti-typhus serum (rabbit, concentrate) (Wyckoff and Bohnel, 1942)	<i>R. prowazeki</i> from egg-yolk sac. European louse-borne strain	1:30
Horse 1	<i>Proteus</i> OX 19 alkali-treated	1:60,000
" 2	"	1:40,000
" 3	"	1:20,000
" 4	"	1:5,000

This difficulty may possibly be overcome by producing the O antibody by immunization with the O antigen of *Proteus* X 19. Horses are now being immunized at the serum depart-

ment of the Lister Institute at Elstree in collaboration with Dr. G. F. Petrie. The immunizing material was designed to contain in concentrated form the alkali-stable O antigen of *Proteus* X 19, which, according to Bruce White (1933), is common to both *R. prowazeki* and *Proteus* X 19. Table II shows the OX 19 titres of the serum from horses immunized in this way. For comparison the OX 19 titres of the therapeutic sera prepared by Durand and Balozet (1940) and by Wyckoff and Bohnel (1942) have also been included in the table.

Three of the four horses immunized with OX 19 antigen developed titres higher than those usually met with in recovered typhus patients. The titres also compare favourably with the titre of the typhoid O antibody in therapeutic anti-typhoid serum (Felix and Petrie, 1938). It is known that the strong antitoxic activity of anti-typhoid Vi + O serum is mainly due to its anti-O component, and it seems worth while trying whether a potent OX 19 immune serum will exert a similar effect in cases of typhus fever.

It is hoped that a supply of this serum will shortly be available for clinical trial along with anti-rickettsia serum. Evidence of the efficacy of such treatment cannot be obtained from animal tests, since none of the experimental animals develops toxic symptoms comparable to those of louse-borne typhus in man. Anti-OX 19 serum should, of course, be tried in combination with anti-rickettsia serum containing neutralizing antibody in high titre. The relative importance of the effects of each of the two antibodies on the course of the disease can be assessed only by clinical trials on typhus patients.

Prophylactic Immunization

Immunization against louse-borne typhus is still in the experimental stage. It is only two or three years since methods of growing rickettsiae in large quantities have been developed. Vaccine made from the gut of infected lice (Weigl, 1930) is the only type of killed typhus vaccine that has, so far, passed its test for efficacy in the field. Unfortunately this vaccine is too difficult to make on a large scale. Most of the workers in America favour the use of rickettsiae grown in the yolk sac of developing chick embryos (Cox, 1938); French workers follow the method of Castaneda (1939) and grow the rickettsiae in the lung of infected mice or rabbits (Durand and Giroud, 1940). In Germany all three techniques are apparently employed.

A number of other problems of importance still remain to be solved before typhus vaccines of maximum prophylactic value can be obtained. One of these is the question how the labile rickettsial antigen can best be maintained in the cultures. The best methods of killing and preserving the vaccines have also yet to be found. At present phenol and formalin are employed. It is known that typhoid vaccines prepared in this way do not contain the labile Vi antigen in its most efficient form (Felix, Rainsford, and Stokes, 1941), and it is quite probable that the labile rickettsial antigen also is damaged by phenol and formalin.

The customary methods of testing the efficacy of typhus vaccines are active immunization of guinea-pigs and tests for OX 19 and neutralizing antibodies in the serum of inoculated subjects. It is not advisable to rely on any one of these tests as the sole method of assay. All three types of test should be employed when differently prepared vaccines are compared.

Tests of OX 19 agglutinin response were recently carried out on a number of volunteers in this country. The subjects were members of selected hospital staffs and sanitary personnel who were being inoculated under a scheme organized by the Ministry of Health in consultation with the Medical Research Council.* The immunizing course consisted in each instance of three doses of the size recommended by the various laboratories issuing the vaccines. Each volunteer had a sample of blood withdrawn before receiving the first dose of vaccine; a second sample was taken a fortnight after the third dose. Any rise in OX 19 agglutinin titre of 100% or more was taken to be a "significant" increase in antibody content. The results are summarized in Table III.

The table shows that the antigenic value of the various vaccines, as judged by the O antibody response, differed very much. Rabbits injected intravenously with the same vaccines failed to disclose any significant difference; all responded with OX 19 antibody formation.

TABLE III.—Showing the OX 19 Agglutinin Response in Volunteers Inoculated with Various Typhus Vaccines. (Nov., 1941, to March, 1942)

Batch	Vaccines		Number Inoculated	Number and Percentage showing Significant Rise in OX 19 Agglutinin Titre	
	Prepared from Rickettsiae grown in	Prepared or Issued		No.	%
A	Yolk sac. European louse-borne strain	Oct., 1940	68	3	4.4
B	" " "	10/5/41	144	25	17.3
C	" " "	4/11/41	125	62	49.6
D	Mouse lung. Tunisian louse-borne strain. (Dr. G. M. Findlay)	1/11/41	16	3	18.7
E	Rat lung. American murine strain	Date of expiry, April, 1943	12	0	0

Neutralization tests of the sera from the various vaccine groups have not yet been carried out, but it is hoped the results will be available for publication at a later date. It is possible that phagocytosis tests may eventually be employed for the quantitative estimation of the antibody to the labile rickettsial antigen, since the O antibody, according to Castaneda (1936a), does not take part in the opsonization of *R. prowazeki*.

Recent reports from Germany emphasize the fact that a death from louse-borne typhus has not yet been recorded in a patient who had been fully immunized with Weigl's vaccine. Very encouraging results also have been obtained in Rocky Mountain spotted fever, the most malignant variety of typhus, by the use of a similar vaccine prepared from infected ticks. Parker (1941), summarizing the results of 15 years' experience of his tick vaccine, showed that the case-fatality rate was 82.3% in non-vaccinated cases in adults but only 8.1% in fully vaccinated cases. Unsuccessful vaccination experiments of earlier workers seemed to suggest that the rickettsiae in the arthropod vector may have some peculiar properties which rickettsiae in the tissues of man and mammals do not possess. It now appears to be likely that the differences are quantitative rather than qualitative: If this is so, one is justified in expecting *R. prowazeki* from animal tissue to yield vaccines of an efficacy equal to that of Weigl's louse vaccine.

Summary

The serological diagnosis and the classification of the fevers of the typhus group are based, so far, on the heat-stable O antigen which is common to the various types of *Rickettsia* and the corresponding types of *Proteus* X. Further subdivision will be made possible by the study of the heat-labile rickettsial antigen.

The roles of the two rickettsial antigens in typhus immunity are very similar to those played by the Vi and O antigens in immunity to typhoid fever.

Therapeutic anti-typhus sera, as at present prepared, contain only negligible amounts of the O antibody. The use of *Proteus* OX 19 immune serum, in combination with anti-*R. prowazeki* serum, is suggested for the treatment of cases of louse-borne typhus.

The methods of preparing and testing typhus vaccines are discussed.

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* Ministry of Health Memo. 252/Med., p. 3, para. 4.

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CIRCUMCORNEAL INJECTION AS A SIGN OF RIBOFLAVIN DEFICIENCY IN MAN WITH AN ACCOUNT OF THREE CASES OF ARIBOFLAVINOSIS

BY

HAROLD SCARBOROUGH, M.B., Ph.D.

(From the Clinical Laboratories and Medical Out-patient Department of the Edinburgh Royal Infirmary)

From the heterogeneous collection of signs and symptoms called pellagra a number of specific syndromes are gradually becoming distinct. In 1938-9 Sebrell and Butler first described the specific syndrome of riboflavin deficiency in man, characterized by undue redness of the buccal mucous membrane at the angles of the mouth, by softening of the skin and subsequent fissuring at those angles, and by reddening, fissuring, and desquamation of the vermilion areas of the lips. Similar lesions were observed in the vestibule of the nose and involving the alae nasi and naso-labial folds; and seborrhoeic lesions of the nose, eyelids, and ears were frequent. These findings were confirmed by, among others, Sydenstricker, Geeslin, Templeton, and Weaver (1939), who drew attention to an associated dermatosis which was seen in 2 cases out of 6. A skin lesion, apparently similar, has since been noted by Duckworth (1942). In 1940 Kruse, Sydenstricker, Sebrell, and Cleckley added a further sign—viz., a specific glossitis, different from the red atrophic tongue of the pellagrin in that the organ is purple or magenta in colour, clean, with "flattened or mushroom-shaped" papillae and frequently fissured. They described for the first time ocular lesions characterized by circumcorneal injection, conjunctival congestion, and keratitis. Vascularization of the cornea followed by infiltrations and opacities had previously been described in ariboflavinosis in rats by Bessey

and Wolbach (1939). All the above stigmata have been repeatedly resolved by riboflavin but not by any other vitamin, and all except the dermatoses have been produced as the result of experimental feeding.

The ocular manifestations of ariboflavinosis were more fully described by Sydenstricker, Sebrell, Cleckley, and Kruse (1940), who found by means of slit-lamp examination of the cornea that a superficial vascularizing keratitis was the principal ocular manifestation of ariboflavinosis in man. Definite ocular changes could be observed in subjects presenting no evidence of cheilosis or glossitis. Photophobia, blepharospasm, dimness of vision, burning sensations in the eyeballs, "roughness" of the eyelids, and extreme visual fatigue were frequent symptoms, and impairment of visual acuity in the absence of any refractive error or opacity was also described. The earliest and most common ocular sign was found to be circumcorneal injection, which, though "often grossly visible," could always be discerned with the slit-lamp microscope, which revealed actual invasion of the cornea by capillaries in 37 out of 47 cases. Gross injection of the vessels of the fornix and sclera without evidence of infection was also common (43 out of 47 cases). Certain changes in the iris (frank iritis, congestion and pigmentation of the iris) were observed, but much less frequently. Many of these lesions resemble, but are actually distinct from, the ocular manifestations of avitaminosis-A.

Among the series of 47 cases described by Sydenstricker *et al.* (1940) was a group of 18 apparently well-nourished institutional employees, 14 of whom were between the ages of 20 and 35. Only 3 of them were taking "restricted" diets on account of intercurrent disease. All these subjects were considered to have circumcorneal injection, and in addition 13 showed evidence of corneal vascularization when examined with the slit-lamp. On account of this striking finding, but also because of the relative ease with which circumcorneal injection may be detected, it was thought worth while to determine the incidence of this sign in a series of individuals of the hospital class. The results of such an investigation are now presented because of their striking nature, because we believe that the experience of other workers will have been similar, and in order to draw attention to the care which is necessary before circumcorneal injection can be ascribed to ariboflavinosis.

Material and Methods

The investigation was undertaken on a series of 204 cases seen during the routine work of the medical out-patient department of the Edinburgh Royal Infirmary between Oct., 1941, and March, 1942—i.e., during the season when the highest incidence of deficiency disease might be expected to occur. The ages of the patients, none of whom suffered from any ophthalmic infection, varied from 12 to 69. Each subject was

Chart showing incidence of circumcorneal injection, in reference to age, among 204 cases.

examined for circumcorneal injection according to the description given by Sydenstricker and his colleagues, but in no case was the slit-lamp used, and no detailed examination for other stigmata of vitamin deficiency disease was made except in 8 selected cases referred to later. The great majority of the patients suffered from chronic organic disease, but, so far as is