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**FOURTH
WHO SCIENTIFIC GROUP
ON TRACHOMA RESEARCH**

Report

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FOURTH WHO SCIENTIFIC GROUP ON TRACHOMA RESEARCH

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FOURTH WHO SCIENTIFIC GROUP ON TRACHOMA RESEARCH

Report

A WHO Scientific Group on Trachoma Research was convened in Geneva from 9-14 August 1965 to advise the Director-General on developments and research needs in this field. The meeting was opened by Dr F. Grundy, Assistant Director-General. Professor H. Bernkopf was elected Chairman and Professor Barrie Jones and Dr L. H. Collier Rapporteurs.

INTRODUCTION

Since the last WHO Scientific Group on Trachoma Research met in 1963 more progress has been made towards clarification of the relationship between trachoma and inclusion conjunctivitis and between TRIC agents and other members of the psittacosis-lymphogranuloma-trachoma (PLT) group of organisms. Further refinements in laboratory diagnostic techniques have facilitated the study of various types of TRIC oculogenital infections and have potential value in the identification of the mild, atypical forms of trachoma that are appearing in areas under collective treatment and that may occur as a result of vaccination programmes. The recent development of techniques for primary isolation of TRIC agents in cell cultures is expected to open up new approaches to several outstanding problems. Although no major advances have been reported in trachoma vaccination studies during the past two years, there are hopes that increasing fundamental knowledge of the PLT agents, of both human and non-human origin, will lead to the production of more effective vaccines. The recent establishment of the WHO International Reference Centre for Trachoma is an important and timely event and will contribute much to the advancement of collaborative studies in a wide range of related problems.

1. PRESENT STATUS OF LABORATORY METHODS FOR THE DIAGNOSIS OF TRIC INFECTIONS

1.1 Isolation of TRIC agents

1.1.1 *Chick embryos*

Recent experience in different laboratories has revealed several points of technical interest. In one comparative experiment, hens' eggs from Gambia appeared more susceptible to TRIC infection than eggs from London. An increase in the susceptibility of eggs to a given dose of the same trachoma strain has been observed in Milan at the same time as a decrease was observed in Ethiopia, and *vice versa*. It was agreed that success in primary isolation is enhanced by use of eggs from antibiotic-free flocks and by very careful control of temperature and humidity in all parts of the incubator. It is particularly important to maintain the embryos as nearly as possible at 35°C after inoculation.

The possibility was discussed that deep scraping of conjunctivae may lead to the collection of blood, and hence of serum antibodies in the inoculum. Most members of the Scientific Group preferred metal scrapers to cotton swabs but it was agreed that, as far as possible, the admixture of blood with scrapings should be avoided.

1.1.2 *Cell cultures*

Cell culture techniques for the rapid primary isolation of TRIC agents are being evaluated in several laboratories. A new method of isolation and serial cultivation using irradiated McCoy human synovial cells was recently reported; the success rate for primary isolation was similar to that for chick embryos. However, subculture from infected cells to the chick-embryo yolk-sac yielded irregular results; there was only slight correlation between the inclusion count in the monolayers and the severity of infection induced in the chick embryo. Further development of the method for use with the TRIC agents was recommended by the Scientific Group.

1.2 Demonstration of inclusions

Technical points of importance were brought out in the review of current methods for demonstrating inclusions:

Iodine staining. If the surface of the specimen on the slide is gently dried with filter paper and then scanned using approximately 200 × magnification, the results are better than with "wet" preparations under a coverslip. A thin film of immersion oil may be used to advantage.

Fluorescent antibody (FA) staining. This technique is being increasingly used. At least two laboratories have reported that when sera are absorbed to reduce non-specific staining and when counterstains are used, the results are as good as, or better than, those with the Giemsa method.

A comparison of the iodine, Giemsa and fluorescent antibody methods in replicate scrapings from the same patients is highly desirable. It is best to examine the entire area of the scraping; this is facilitated by spreading conjunctival specimens as evenly as possible over a definite small area of the microscope slide. For Giemsa stain, the thinnest possible smear is desirable, but for the iodine and FA methods, thick smears increase the chances of finding inclusions quickly.

1.3 Serological methods

1.3.1 *Fluorescent antibody technique*

Improvements in this technique are being made by several groups. Recent work suggests that higher antibody titres are obtained by the indirect FA test than by the complement-fixation (CF) test.

The FA test has been used by two laboratories to distinguish psittacosis from the lymphogranuloma venereum (LGV) and TRIC agents. Furthermore, using cross-absorbed sera the test demonstrated two types of TRIC agent corresponding approximately to those elicited by the mouse toxicity protection test; the similarity of a strain of lymphogranuloma venereum to the Bour and T'ang strains of trachoma, and to the IC Cal-3 strain of inclusion conjunctivitis was also shown. Detection of antibody in fluid in the conjunctival sacs of trachoma patients by FA procedures was considered of particular interest.

1.3.2 *Complement fixation tests*

In tests on sera from trachoma patients, use of boiled phenolized group antigen derived from trachoma agent elicited a higher percentage of positive reactions and higher antibody titres than did group antigen from psittacosis or LGV agents. Similar findings were reported with purified elementary body antigens.

Examination of serial serum specimens taken during the course of clinical trachoma has frequently demonstrated a rise in titre of CF antibodies to group antigen, but there are also many instances in which such changes have not been observed. There is no evidence that CF antibodies are related to neutralizing properties.

It should be emphasized that although CF tests using group antigen are easy to perform and yield reproducible results, they measure only antibodies to the PLT group as a whole; for diagnostic purposes a specific, sensitive and easily prepared antigen is much to be desired.

1.3.3 *Neutralization tests*

Two laboratories have obtained evidence that sera from immunized animals reduce the count of inclusion bodies in cell cultures infected with TRIC agents, and similar findings have been reported with undiluted sera from three volunteers infected by the conjunctival route. A reduction in infectivity was also observed when a TRIC agent was incubated with serum from an immunized rabbit and injected into suckling mice, but sera from trachoma patients yielded negative results. With few exceptions, it has been found difficult to demonstrate neutralization in chick embryos.

1.3.4 *Microagglutination*

Microagglutination of purified elementary bodies has been observed by both darkfield and ordinary light microscopy, using sera from immunized animals and those from trachomatous patients. However, some sera from subjects free from trachoma induced non-specific clumping of the elementary bodies. The specificity of this test might be improved by using more highly purified elementary body suspensions.

Using sera from immunized animals, a fairly clear-cut differentiation has been reported between trachoma and ornithosis. If perfected, it is possible that this test could be useful in serological surveys and as an aid in identifying agents of the PLT group.

1.4 **Delayed skin reactions**

Immunization with TRIC agents may induce in both man and animals delayed hypersensitivity as shown by dermal reactions to both boiled group antigens and purified elementary body antigens. Reactions of this nature were observed more frequently and were more severe in trachomatous subjects in Eritrea than in normal subjects in Italy.

The non-specific reactions frequently observed are possibly due to egg material present in the skin-test antigens. More highly purified reagents might solve this problem. At present, there is still hope for a specific trachoma skin-test antigen for use as a diagnostic aid in man.

2. RELATIONSHIPS BETWEEN TRACHOMA, INCLUSION CONJUNCTIVITIS AND OCULAR LYMPHOGRANULOMA VENEREUM AND BETWEEN AGENTS ISOLATED FROM THESE SYNDROMES

It was agreed that very valuable new observations had been made since the last WHO Scientific Group on Trachoma Research met. It was noted that several volunteers inoculated with strains that had been isolated from

typical cases of inclusion conjunctivitis and grown in the yolk sac developed punctate keratitis and micropannus, which are not characteristic of the classical syndrome. It was agreed that trachoma sometimes heals spontaneously leaving scars and pannus of such slight extent as to be detected only by skilled use of the slit-lamp biomicroscope. Several observers claim that, even when untreated, trachoma may subside without detectable sequelae in the cornea or conjunctiva.

A summary of the biological and epidemiological differences between the three syndromes is presented in the table below.

BIOLOGICAL AND EPIDEMIOLOGICAL DIFFERENCES BETWEEN TRACHOMA, INCLUSION CONJUNCTIVITIS AND OCULAR LYMPHOGRANULOMA VENEREUM

Distinguishing feature	Syndrome in which present		
	Trachoma	Inclusion conjunctivitis	Ocular LGV
1. Follicles	Yes	Yes	No ^a
2. Conjunctival scars	Yes	No	Yes ^a (severe)
3. Corneal scars	Yes	No	Yes ^a
4. Gross pannus	Yes	No	Yes ^a
5. Micropannus	Yes	Yes ^b	Yes
6. Cause of blindness or impairment of vision	Yes (important)	No	Rare ^c
7. Genital source of infection	More data needed	Usual	Almost always
8. Eye-to-eye transmission	Usual	Rare	No data
9. Microscopic findings in conjunctival scrapings:			
Inclusions in epithelial cells	Yes	Yes	No
Carbohydrate matrix	Yes	Yes	No
Inclusions in monocytes	No	No	Yes
10. Primary isolation of agent:			
In mouse brain	No	No	Yes ^d
In cell culture	No ^e	No	Yes ^a
11. Frei test (antigen derived from egg-propagated LGV)	No	No	Regularly positive ^d

^a Based on a small number of cases recorded in the literature.

^b Has been observed recently; frequency of occurrence remains to be determined.

^c Blindness has been caused by LGV but cases very few in number.

^d Based on genital infections with LGV, in addition to the few ocular cases reported.

^e Reported successful in one laboratory; not yet confirmed elsewhere.

The data summarized in this table have been cited as evidence for postulating the existence of biologically different causative agents for trachoma, for inclusion conjunctivitis, and for lymphogranuloma venereum.

Another interpretation of the various observations is that there may be several causative agents involved in each of the three clinical syndromes,

particularly as the trachoma syndrome has recently been demonstrated following inoculation of material derived from the genital tract. One observer suggested that separate recognition be accorded to cases of follicular conjunctivitis associated with a TRIC agent in which focal corneal infiltrates often occur, sometimes in the central area of the pupil, without pannus, and without permanent damage.

The relation of LGV to inclusion conjunctivitis and trachoma was considered by the Scientific Group. It was noted that invasiveness and pathogenicity of LGV for mice by primary intracerebral inoculation serve to distinguish LGV from inclusion conjunctivitis and trachoma in the laboratory, and that the occurrence of clinical disease in the eye caused by LGV should not cause serious difficulties in diagnosis. The Scientific Group agreed, however, that before labelling inclusion-producing isolates from either the male or the female genital tract as TRIC organisms as opposed to LGV, and before proceeding with inoculation of volunteers, the isolates should be characterized by currently available laboratory tests¹ to ensure that they do not have the invasive properties of LGV.

It was recognized that in some cases the presenting symptom may be punctate keratoconjunctivitis due to TRIC agents, but the relation of these cases to inclusion conjunctivitis and trachoma remains to be elucidated. The Scientific Group agreed that agents isolated from such cases should not be designated at present either as inclusion conjunctivitis or as trachoma.

The Scientific Group was unanimous in recommending continued research on the PLT organisms isolated from ocular and genital sites, with emphasis on their biological characteristics and on new laboratory methods for rapid and reliable recognition of their distinctive properties.

2.1 Relevance of clinical differential diagnosis to field studies

The problems relating to the clinical definition of trachoma, inclusion conjunctivitis and inclusion blenorrhoea (and even LGV conjunctivitis) do not affect field trials of trachoma vaccine. These studies are carried out in areas where TRIC infection of the eye is epidemiologically consistent with trachoma and where prevalence and gravity of the disease warrant intervention. The variations from one place to another in the clinical aspects and the course of the disease will, however, affect the criteria used for its diagnosis and for assessing its gravity. A critical analysis of the local clinical, epidemiological and microbiological aspects of the disease before embarking on a vaccine study is therefore imperative for the develop-

¹ Including intracerebral inoculation of both suckling and young adult mice with early egg-passage material, followed by observation for ten days.

ment of a set of criteria. These criteria, if strictly adhered to and kept unchanged during the entire course of the study, will serve as indices for determining a change in the incidence and/or pattern of the disease.

3. STRAIN DIFFERENCES IN TERMS OF MORPHOLOGY, NUTRITIONAL REQUIREMENTS, SEROLOGICAL PROPERTIES AND PATHOGENICITY FOR VARIOUS HOSTS

3.1 Morphology and nutritional requirements

The last WHO Scientific Group on Trachoma Research noted in its report (unpublished) that inclusions induced by TRIC agents differ morphologically from those of psittacosis. This has now been confirmed by both light microscopy and electron microscopy. The electron microscope has also revealed a close similarity between the modes of replication of TRIC and LGV agents.

At least one laboratory strain of psittacosis (6 BC) appears less demanding than TRIC agents in its nutritional requirements in cell culture. It is desirable to extend these observations to a wider range of isolates.

3.2 Serology

3.2.1 *Mouse toxicity protection test.* The Group recommended that the use of the term "neutralization" in connexion with this test be abandoned.

Because of the technical difficulty of the test, the number of strains examined is still limited. The possibility was considered that isolates cross-reacting with representatives of the two main types elicited by the test might derive from mixed infections, but further work is necessary to elucidate this point.

The Group recommended that an attempt be made to agree on nomenclature of the types described by the individual laboratories undertaking this test.

3.2.2 *Immunofluorescence.* By this method TRIC and LGV agents could be clearly distinguished from the 6 BC strain of psittacosis and from an isolate from sheep abortion. Using suitable absorbed sera it has been confirmed that TRIC agents can be divided by immunofluorescence into two serotypes which in general correspond to the types elicited by the mouse toxicity protection test; although the fluorescent antibody method may not discriminate as finely as the mouse test, its comparative simplicity renders it potentially more useful for examining large numbers of strains, and for rapid typing of new isolates.

3.2.3 *Complement fixation tests.* Recent reports confirm that species-specific antigens can, with some difficulty, be detected in members of the

PLT group. There is, however, still no simple method for preparing such antigens, or for titrating species-specific serum antibodies.

3.3 Pathogenicity for chick embryos

Several workers have obtained evidence that some TRIC agents alter in their virulence for chick embryos during repeated passage in the yolk sac. With a given dose, these variant strains, which appear to be mutants, kill the embryos more rapidly than the strains from which they are derived, and the acquisition of this property is associated with the ability to grow readily in cell cultures. There is evidence that the comparatively rapid lethal effect in chick embryos is due to more rapid multiplication rather than to increase in the toxin/particle ratio. One such variant strain has been observed to induce generalized infection in mice after inoculation by various parenteral routes ; by contrast, the limited experiments so far undertaken suggest that these variants are diminished in pathogenicity for the primate conjunctiva. Nevertheless, in view of uncertainty about the pathogenicity of such strains for man, and the possibility that they might cause generalized infection, the Group is of the opinion that such strains should not be inoculated into man until they have been proved susceptible to chemotherapy and/or antibiotic treatment, and devoid of neurotropism for primates when inoculated intracerebrally.

3.4 Pathogenicity for cell cultures

Fast-killing variants of the type described in the paragraph above readily induce inclusion formation in a wide range of cell cultures ; transformed and diploid cell lines and primary explants are all susceptible, and the variant strains can be passaged indefinitely in them. By contrast, slow-killing or "wild-type" TRIC agents will grow in cell cultures only when centrifuged into monolayers, and even then their growth is limited to one or at most a few cycles. An exception to this observation is the recent finding that McCoy human synovial cells treated with gamma radiation readily support the growth of TRIC agents, and can be used for primary isolation. TRIC agents propagated in this way can be passed serially in irradiated cells, but passage of the harvests to chick-embryo yolk sacs yields irregular results. This finding is of obvious importance in relation to improvements in isolation technique, and its early confirmation by other workers is highly desirable.

3.5 Pathogenicity for animals

The last WHO Scientific Group on Trachoma Research called attention to the wide variations in pathogenicity of TRIC agents for the primate conjunctiva ; since then, knowledge of the host range and pathogenicity of

TRIC and allied agents has been extended in several directions. The finding that micro-organisms of the PLT group cause conjunctivitis in guinea-pigs and cats suggests a possible use for these animals as alternatives to primates in the study of eye infections due to these agents. Furthermore, several investigators have obtained serological and morphological evidence linking the LGV agent more closely with TRIC agents than with those of the psittacosis/ornithosis subgroup; the isolation of what appears to be a TRIC agent from the rectum of a patient with proctitis also suggests reconsideration of the previous restricted view of the relationship between these organisms, and calls for further research on their pathogenicity for different tissues.

4. EVIDENCE OF LATENCY IN TRIC INFECTIONS

Latent or subclinical infections have long been recognized in psittacosis and ornithosis in birds and in lymphogranuloma venereum in man. However, latency in the sense in which it is used in connexion with true viruses, such as herpes simplex (when no formed elements can be detected in periods of latency) probably does not exist in infections due to the PLT agents.

In trachoma there are well-documented cases of smouldering disease in which acute exacerbations occurred spontaneously or under the influence of corticosteroids, bacterial infections, trauma and other provocations. In these, however, the evidence suggests that clinical disease of minimal intensity was present in the interval phases. Experimentally it has been well shown that inclusion bodies can often be demonstrated in the incubation period before the onset of clinical disease. Apart from these cases, there are claims in the literature for the demonstration of inclusion bodies in clinically normal conjunctivae, especially in young infants. The Scientific Group felt that these reports must be treated with reserve, since inexperienced workers frequently confuse non-specific cytoplasmic elements, such as melanin granules, with true Halberstaedter-Prowazek inclusions. It recommended further investigation of this important subject.

More important than the question of latency is that of trachoma cases exhibiting few or no symptoms yet serving in a family or a community as a reservoir from which healthy individuals, particularly young children, may become infected. It is obvious that under field conditions, where mild chronic conjunctival inflammation due to dust, wind, sun and bacteria of low pathogenicity is common, trachoma of low intensity will often remain undiagnosed. The Scientific Group felt that an effort should be made to improve the criteria on which the clinical diagnoses of Stages I and IV are made. In view of the sensitivity of the FA method for detecting TRIC agent in the conjunctiva, it was suggested that this method be used

in parallel with clinical observations to determine the reliability of clinical diagnosis of the early and of the healed state.

A recent report from South Africa suggests that trachoma can masquerade under the guise of a chronic catarrhal conjunctivitis, and that isolations have been obtained from Stage IV cases. If these observations are confirmed it would upset present concepts of trachoma diagnosis and control and would make necessary the field use of laboratory aids on a scale not now contemplated. Further investigation is urgently needed.

Epidemiologists who have noted reinfection after apparently successful treatment of schoolchildren have often encountered smouldering trachoma in relatives who are in constant contact with the children. The subject of latent and minimally active trachoma is thus of great epidemiological importance.

Little is yet known of the importance of latency and low activity in inclusion conjunctivitis, but TRIC agent has been isolated from both the urethra and the cervix in patients without symptoms. It is obvious that such cases may be very important in the spread of the agent.

5. TRACHOMA VACCINES

The Group reaffirmed the desirability of developing an effective trachoma vaccine. Since the last WHO Scientific Group on Trachoma Research met, laboratory and field studies have been considerably extended. The general principles laid down by that Group and quoted in the third report of the WHO Expert Committee on Trachoma¹ should be closely observed in any programmes for the development and evaluation of new vaccines.

5.1 Vaccine development programmes

The development of a trachoma vaccine, like that of any other antigen intended for large-scale use, entails a preliminary series of interlinked epidemiological and laboratory studies, followed by pilot field trials. Should the latter prove successful, large-scale field trials in various endemic areas would follow, and might eventually lead to manufacture on a commercial basis.

5.1.1 *Basic requirements*

Vaccines intended for large-scale field use must not provoke general reactions or severe local reactions, or cause sensitization to the antigen itself or to other constituents of the vaccine. A limited number of injec-

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1962, 234, 29.

tions must confer reasonably long immunity against the antigenic varieties of TRIC agent prevalent in the country of use. The vaccine must be easy to store and administer under field conditions in hot countries, and it is obviously desirable that production costs be low enough to make mass distribution economically practicable.

5.1.2 Potency tests

Before subjecting a vaccine to an elaborate and expensive field trial, it is obviously desirable to have some laboratory evidence of potency. Potency tests on established vaccines involve measurement of some characteristic known to be correlated with their power to protect against the naturally occurring infection, for example, capacity to protect a susceptible species against artificial challenge with the virulent organism. In some instances, it is necessary only to do a simple quantitative test ; thus titration of smallpox vaccine in rabbits or in chick embryos eliminates the need for tedious protection experiments. As far as trachoma vaccine is concerned, none of the features that can be tested in the laboratory has so far been definitely correlated with protective effect in the field ; to remedy this situation, it is most important to record as much information as possible about every batch of vaccine used for field trials. The following methods are at present available for assaying TRIC antigens :

(a) *Ability to confer protection against artificial challenge by the conjunctival route.* Since only primates appear to be susceptible to infection by this route, such tests must be done in simian species or in human volunteers. Although these experiments are tedious and expensive, they are, short of an actual field trial, the most direct test of potency available.

(b) *Ability to induce antibody response in animals and man.* Antibodies to TRIC agents can be assayed by complement fixation ; neutralization of infectivity for cell cultures, mice and chick embryos ; agglutination ; and immunofluorescence (direct and indirect methods). Recent findings suggest that antibodies to PLT agents might also be studied by gel diffusion techniques. There is as yet no evidence as to which of these methods is the most useful but, *prima facie*, those based on neutralization of infectivity seem to be the most relevant to the purpose under discussion.

(c) *Content of toxin lethal for mice.* Some investigators have used this assay as an index of suitability for field use, but there is as yet no firm evidence that the results are correlated with protective effect.

(d) *Mouse toxicity protection test.* By prior immunization with a suitable vaccine, mice can be protected against the lethal effect of TRIC agents injected intravenously. It is not known whether the ability of a given vaccine to protect mice against intravenous challenge with homologous antigen is related to its performance in protecting primates against

conjunctival infection ; there is, however, some evidence that the strain grouping elicited by the mouse test is reflected in the results of cross-tests in monkeys challenged by the conjunctival route.

(e) *Content of antigen.* Ultimately, a simple assay of the amount of antigen in a given batch of vaccine would be the most satisfactory index of potency, provided of course that it were directly related to protective effect. At present, the amount of TRIC agent in a given preparation is usually estimated by one or more of the following methods :

- (i) total particle count ;
- (ii) amount of complement-fixing antigen ;
- (iii) amount of live virus, in terms of egg-lethal doses, egg-infective doses and, for strains that grow in cell cultures, inclusion-forming units (in the case of inactivated vaccines, the content of live virus should be estimated immediately before inactivation) ;

One laboratory also estimates the deoxyribonucleic acid content of purified vaccine but the proportions of DNA associated with the agent itself and with impurities are not known.

5.1.3 *Preparation of vaccine*

(a) *Choice of host cell.* The trachoma vaccines so far used in man have been prepared from cultures in chick-embryo yolk sacs, in which all known TRIC agents can be grown and which have the merit of yielding large quantities of antigen. The disadvantages are the possibility of inducing sensitization to egg protein ; relative difficulty in purification ; and, particularly in the case of live antigens, possible contamination with other agents, such as avian leucosis virus, unless specially tested eggs are used. In this connexion note should be taken of the possibility that current stocks of vaccine seed may contain avian leucosis virus.

Although antigens grown in cell cultures have as yet been used only in animal experiments, they are potentially useful for vaccine production. The yield of virus will probably be less than that from eggs, but such preparations are more readily purified and are free from avian leucosis virus. On the other hand, primary explants are liable to carry other contaminants, such as vacuolating agents, which again are a potential hazard in the case of live antigens. The use of diploid cell lines has not so far received official approval because of doubts about the significance of chromosomal anomalies in serial subcultures.

(b) *Choice of vaccine strains.* The TRIC agent(s) to be incorporated in a vaccine must produce a high yield in the culture system employed and must possess satisfactory immunogenicity in relation to the strains prevalent in the country of use ; consideration must be given to the question of using monovalent or polyvalent vaccines.

(c) *Choice of live or inactivated antigen.* Most investigators have used inactivated antigens both for laboratory studies and for field trials. There is, however, some evidence from animal experiments that live antigens are more effective, and such preparations have been used in man without untoward effects. The Group recommended, however, that such vaccines should not be used in man until they have been shown to be free of pathogenicity on injection into the simian brain.

(d) *Tests for innocuity and purity and for freedom from contaminants.* There are as yet no national or international regulations specifically dealing with trachoma vaccine; pending the formulation of such requirements, manufacture and testing of any antigens destined for use in man should conform with the applicable official requirements relating to viral vaccines in the countries in which they are to be manufactured and/or used.

5.1.4 *Conduct of field trials*

As mentioned on page 12, the principles to be observed in the design and conduct of trachoma vaccine field trials were set out in the third report of the WHO Expert Committee on Trachoma. The Scientific Group re-emphasized the absolute necessity for valid randomization and "double-blind" procedures, for preliminary definition of diagnostic criteria, and for strict adherence to these throughout the trial. Should more than one ophthalmologist be involved, mutual agreement on criteria, methods of examination, scoring, and recording of physical signs should be ensured by preliminary testing and standardization.

It is recognized that even if outright protection against infection is not achieved, a vaccination programme might change the character of trachoma in a community from a grave and disabling disease to a milder form with fewer complications. It is therefore essential that the data collected in all field surveys be adequate to permit the detection of any such changes. Similar changes could of course result from factors other than vaccination.

The Group strongly recommended the uniform recording and scoring of physical signs as follows:

(a) *In small-scale studies* where every individual is fully scrutinized with the aid of a major (conventional) biomicroscope and with fluorescein staining of the cornea: all the physical signs related to TRIC ocular infection (see Annex, page 20).

(b) *In large-scale trials* where it is not feasible to submit every individual to a thorough examination: the minimal set of clinical signs recommended by the Group (see Annex, page 23). It was strongly recommended that in a large-scale trial a randomly selected sub-sample be submitted to a thorough examination using the major biomicroscope and fluorescein staining of the cornea.

The Group strongly recommended that detailed tables of the physical signs observed and changes in them be either included in or annexed to reports on both small and large-scale vaccine trials.

5.2 The present status of field studies

A number of workers have reported that both live and inactivated TRIC antigens protect human volunteers and simians against a challenge dose of the agent administered by the conjunctival route comparatively soon after the completion of vaccination. However, reports about the performance of trachoma vaccines under field conditions are conflicting. It is difficult to compare the findings of different groups of workers because of differences in the local epidemiology of trachoma and intercurrent eye infections; in the age-groups selected for vaccination; in methods of immunization; and in systems for recording and assessing the results. The main conclusion that emerges from the various reports is that TRIC agents are comparatively poor antigens, and that a fully effective vaccine is not yet available. Although a measure of protection can be obtained against both experimental and naturally occurring trachoma and there is some evidence of therapeutic effect, there is no assurance of solid immunity; several reports suggest, moreover, that any beneficial effect is of comparatively short duration. Nevertheless, very considerable progress is being made in fundamental knowledge of the PLT group, and there is hope that improved vaccines will result.

5.3 Further research

The Group considered that further studies are required on the following aspects of trachoma vaccine research:

- (a) Development of a suitable laboratory test of potency; in this connexion, it is noteworthy that the level of complement-fixing antibody induced by vaccination appears to bear little or no relationship to immunity.
- (b) Nature of the immunizing antigen of TRIC agents; possible use of antigenic fractions for immunization.
- (c) Enhancement and prolongation of artificially induced immunity, including further investigations of the action of adjuvants, about which there are conflicting reports.
- (d) Importance of various antibodies in immunity.
- (e) Further comparisons between live and inactivated antigens.
- (f) Strain variation in immunogenicity, and extent of cross-protection against conjunctival infection by defined serological types of TRIC agent.
- (g) Minimum dose necessary for immunization, and comparison of different vaccination schedules and routes of injection.

(h) Possible occurrence of sensitization to various constituents of vaccines, with particular reference to the possible role of sensitization or incomplete immunization in the production of pannus or corneal opacities of the "nummular" or "punctate" type.

(i) Consideration of various host tissues for production purposes, with particular reference to the problem of making leucosis-free vaccine from eggs, and to the merits and disadvantages of diploid cell lines and primary cell cultures.

(j) Shelf life of vaccines stored in the liquid, frozen, or dried state.

5.4 Publicity

There is a distinct possibility that unconfirmed and over-optimistic reports through public information media may influence health authorities to relax prematurely their efforts to control trachoma by means other than vaccination. While recognizing that research on trachoma vaccine is a matter of great public interest, the Group strongly recommended that workers in this field use their best endeavours to prevent the appearance of misleading publicity of this nature.

6. TRACHOMA THERAPY

6.1 Comparative testing of substances known or suspected to have therapeutic value in trachoma

Laboratory testing of antibiotics has been undertaken since the trachoma agent was first isolated; the techniques have since been developed and refined, using both chick embryos and cell cultures.

The two systems have yielded similar and reproducible results, which in turn have largely paralleled clinical experience. For example, the superiority of tetracycline over chlortetracycline in laboratory tests has been confirmed in controlled field trials. However, the correlation is not complete. Some laboratory workers have reported erythromycin to be more active than the tetracyclines whereas others found the reverse. In two independent field trials, erythromycin was found to be slightly but significantly superior to tetracycline. This subject requires further research and standardization of techniques.

6.2 Possibility of induced resistance of TRIC agents to antibiotics

Comparative trials were undertaken over a period in two areas where trachoma is endemic and where large-scale treatment campaigns with antibiotics have long been pursued; in the most recent trials, the cure

rate was disappointing. The Group agreed that the possibility of induced resistance to antibiotics calls for immediate investigation and for continued vigilance in the future. Although final proof of change in the efficacy of drugs must depend on well-controlled clinical trials, such investigations are difficult, time-consuming and expensive; 500-600 cases are needed in each treated group and in each control group in order to establish a statistically valid 10% difference in cure rates; there are, moreover, few endemic areas where reliable data are available on cure rates when antibiotics were first used, and fewer with yet untreated populations where expert teams are available to conduct base-line studies. Discrimination is clearly needed in utilizing these limited field resources.

It is therefore imperative that laboratory methods be used for determining levels of and changes in sensitivity of TRIC strains to antibiotics and other drugs. The use of laboratory techniques for testing the relative efficacy of different antibiotics presents no serious problems, since for this purpose readily cultivable TRIC strains of constant pathogenicity are available. It may, however, be difficult to develop methods for testing the relative drug sensitivity of a variety of fresh TRIC isolates, whose characteristics and behaviour in culture cannot be foreseen. In this connexion, the Group noted that it may now also be possible to test the antibiotic sensitivity of freshly isolated strains in irradiated cell cultures (see section 1.1.2).

The Group recommended that high priority be given to both the clinical and the laboratory aspects of this problem, particularly in view of the large-scale treatment programmes now in operation in many parts of the world.

7. WHO INTERNATIONAL REFERENCE CENTRE FOR TRACHOMA

The Group particularly welcomed the establishment of the WHO International Reference Centre for Trachoma which was due to begin operation on 1 September 1965 in the laboratories of the Francis I. Proctor Foundation for Research in Ophthalmology of the University of California Medical Center in San Francisco. Emphasis will be placed on the collection of materials and data, storage and distribution of reference strains, production of antisera, exchange of information, and the initiation of collaborative research and training. The early distribution of significant papers to selected laboratories will be attempted, possibly by means of an information exchange group, similar to the group sponsored by the National Institutes of Health, Bethesda, USA. It is expected that at least twelve months will be required for the Centre to reach full activity.

8. RECOMMENDATIONS FOR FUTURE ACTION BY WHO

The Group recommended that WHO should continue and, as far as possible, extend its activities in relation to the training of scientific personnel, the support of research on the biology of TRIC agents, and the carrying out of multi-disciplinary field studies to provide the type of information required for the development and evaluation of prophylactic and therapeutic measures.

It was suggested that the WHO International Reference Centre for Trachoma might advise workers in other institutions of the occurrence of epidemics of follicular conjunctivitis, including those possibly associated with parakeets or other animal species, and organize collaborative investigation of such outbreaks.

The training of microbiologists, epidemiologists, ophthalmologists and laboratory technicians was considered to be of great importance. Such training can be of short duration where experience in a single technique is needed, or for periods of a year or more to enable a worker to be trained in research and to carry out a specific investigation. WHO's programme of fellowships, research training and exchange of scientific workers should continue to be applied to these needs.

The Group recommended that WHO should continue its support of studies of therapeutic agents and treatment schedules suitable for mass application, and should sponsor collaborative laboratory and clinical investigations into the possible occurrence in certain areas of induced resistance to TRIC agents to antibiotics.

It was further recommended that WHO should consider whether studies in immunopathology might usefully be applied to elucidating the pathogenesis of trachoma and whether ecological methods might be employed in studying the distribution of the disease. The possible contributions of studies on PLT agents of non-human origin should also be borne in mind. The criteria that have been established for the conduct of trials of trachoma vaccine should be kept under review.

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Annex

FIELD STUDIES IN TRACHOMA

Methods of examination, scoring and recording of physical signs

1. Possible types of examination and combinations to be used in different circumstances

- (a) Naked eye
- (b) Binocular loupe up to $5\times$ magnification with focal illumination
- (c) Monocular loupe $7.5\times$ or more magnification with focal illumination
- (d) Major (conventional) slit-lamp biomicroscope

Most trials are based on the use of method (a) or (b), or on a combination of methods (a) and (c), (b) and (c), (a) and (d) or (b) and (d).

Trachoma in its natural state is practically always bilateral and usually symmetrical. It may be desirable in some studies to record the physical signs for each eye separately but if this is not done and if the physical signs are dissimilar in the two eyes the score recorded should be that of the more severely involved eye.

It should be noted that trials may be based on the observation of only right or only left eyes, provided that this is decided at the time of the initial examination.

The diagnosis by stage of trachoma must be made in each case and at each examination but only after recording all the physical signs.

It is of the greatest importance that details of the methods of examination used, and the signs looked for and recorded in any trial should be given in full when reporting the results.

2. Methods of scoring and recording

A. *Full examination* (using available diagnostic aids: major biomicroscope and staining of cornea) practicable only in detailed studies of small groups.

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE ¹
<i>Ptosis</i>	Detectable	1
	Definite, but not completely covering the pupil	2
	Covering the pupil	3
<i>Sinuuous outline of the upper lid border</i> (Herbert's sign)	Present	1

¹ If the physical sign has been looked for but not detected this should be indicated by a "O"; if the physical sign has not been checked (for example, pannus in very young children) this should be denoted by a blank.

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE
<i>Exudate</i> (observed without previous cleaning)	Minimal	1
	Moderate, but lids not stuck together	2
	Lids stuck together	3
<i>Pre-auricular lymph nodes</i> Size	Just palpable.	1
	Easily palpable (but not detected by observation).	2
	Visibly enlarged	3
Tenderness	Slight, elicited on palpation	1
	Moderate, elicited on palpation (patient resenting palpation).	2
	Severe, associated with pain	3
<i>Hyperaemia of bulbar conjunctiva</i>	Circumcorneal flush or slight extension from the fornices	1
	Patchy.	2
	Total, with or without minute haemorrhages.	3
<i>Oedema of upper lunula</i> ¹ (Wilson's sign)	Detectable by major biomicroscope.	1
	Detectable by the naked eye or binocular loupe	2
	Bulbar chemosis	3
<i>Conjunctival follicles</i> ² Upper tarsal conjunctiva	Involving less than 1/3 of the surface area	1
	Involving 1/3 to 2/3 of surface area	2
	Involving the entire surface area (not confluent)	3
	Total confluent involvement (Stellwag's sign)	4
Upper fornix	Slight involvement	1
	Moderate or marked involvement but not confluent.	2
	Total confluent involvement	3
Semilunar folds	Slight involvement	1
	Moderate or marked involvement but not confluent	2
	Total confluent involvement	3
Bulbar conjunctiva	Presence of follicles.	1
Lower tarsal conjunctiva and fornix	Involving less than 1/3 of the surface area	1
	Involving 1/3 or more of surface area short of total involvement	2
	Involving the entire surface area	3

¹ The term "upper lunula" is used to denote the crescentic semi-opaque zone at the limbus extending from 9 o'clock to 3 o'clock with its widest portion, up to 2.5 mm, at the 12 o'clock position (Busacca, A. (1952) *Biomicroscopie et histopathologie de l'œil*, Zurich).

² These scores apply to immature follicles. It is also necessary to record the presence of mature follicles. If these are present the follicle score given for the site will be multiplied by the factor of 2.

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE
<i>Diffuse cellular infiltration and papillary hyperplasia (upper tarsal conjunctiva)</i>	Minimal ; major biomicroscope needed for recognition, normal vessels not obscured . . .	1
	Moderate ; recognizable by the naked eye or binocular loupe, normal vessels appear hazy.	2
	Pronounced ; conjunctiva thickened and opaque, normal vessels obscured	3
<i>Conjunctival scars</i>	Deviation of upper tarsal conjunctival vessels, and/or fine scattered superficial scars in upper tarsal conjunctiva, or scars of any severity or extent in other conjunctival sites	1
	Moderate readily recognizable scarring with no shortening or distortion of the upper tarsus .	2
	Dense scarring of the upper tarsal conjunctival tissue	3
	Trichiasis and/or entropion	4
<i>Limbus and cornea :</i>		
<i>Pannus</i>		
Vessels (measured from the upper limbus)		
Micropannus	0.5 - < 1.0 mm extension beyond normal limbal opacity, as demonstrated by direct focal illumination	1
	1.0 - < 2.0 mm extension	2
Macropannus	2.0 - < 4.0 mm extension	3
	4.0 - < 6.0 mm extension	4
	6.0 mm or more extension	5
Infiltration just beyond corneal vessels	Minimal infiltration recognizable only by major biomicroscope.	1
	Infiltration barely recognizable by naked eye or binocular loupe.	2
	Dense opacification	3
<i>Limbal follicles</i>	One to three typical follicles.	1
	More than three, but not involving entire upper lunula	2
	Entire upper lunula involved.	3
	Cornea encircled or two rows of follicles above	4
<i>Herbert's pits</i>	One to three typical pits	1
	More than three but entire upper lunula not involved	2
	Entire upper lunula involved.	3
	Cornea encircled or two rows of pits above .	4

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE
<i>Corneal scars</i>	Minimal, resulting in slight or no visual loss ¹	1
	Moderate visual loss, pupillary area involved	2
	Resulting in gross visual loss in one eye	3
	Resulting in gross visual loss in both eyes (economic blindness)	4

B. *Minimal examination* (using naked eye or binocular loupe) practicable in large-scale studies.

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE
<i>Conjunctival follicles</i> ² Upper tarsal conjunctiva	Involving less than 1/3 of the surface area	1
	Involving 1/3 to 2/3 of surface area	2
	Involving the entire surface area (not confluent)	3
	Total confluent involvement (Stellwag's sign)	4
<i>Diffuse cellular infiltration and papillary hyperplasia</i> (upper tarsal conjunctiva)	Moderate; recognizable by the naked eye or binocular loupe, normal vessels appear hazy	2
	Pronounced; conjunctiva thickened and opaque, normal vessels obscured	3
<i>Conjunctival scars</i>	Deviation of upper tarsal conjunctival vessels, and/or fine scattered superficial scars in upper tarsal conjunctiva, or scars of any severity or extent in other conjunctival sites	1
	Moderate readily recognizable scarring with no shortening or distortion of the upper tarsus	2
	Dense scarring of the upper tarsal conjunctival tissue	3
	Trichiasis and/or entropion	4
<i>Limbus and cornea :</i>		
<i>Pannus</i> (Macropannus) Vessels (measured from the upper limbus)	2.0 - < 4.0 mm extension	3
	4.0 - < 6.0 mm extension	4
	6.0 mm or more extension	5
Infiltration just beyond corneal vessels	Infiltration barely recognizable by naked eye or binocular loupe	2
	Dense opacification	3

¹ Assessed by objective examination.

² These scores apply to immature follicles. It is also necessary to record the presence of mature follicles. If these are present the follicle score given for the site will be *multiplied by the factor of 2*.

PHYSICAL SIGN	DEGREE OF INVOLVEMENT	SCORE
<i>Limbal follicles</i>	One to three typical follicles	1
	More than three, but not involving entire upper lunula	2
	Entire upper lunula involved	3
	Cornea encircled or two rows of follicles above	4
<i>Herbert's pits</i>	One to three typical pits	1
	More than three but entire upper lunula not involved	2
	Entire upper lunula involved	3
	Cornea encircled or two rows of pits above .	4
<i>Corneal scars</i>	Pupillary area involved, moderate visual loss ¹	2
	Resulting in gross visual loss in one eye . .	3
	Resulting in gross visual loss in both eyes (economic blindness)	4

Note : In some trials it may be desirable to include the observation of certain other signs, e.g., follicles in the upper fornix or elsewhere.

¹ Assessed by objective examination.
