INTRODUCTION

Current estimates suggest that more than 400 millions of the world's population still suffer from trachoma and its grave sequelae of visual incapacity leads to huge economic loss, particularly in the under-developed countries. For many years, the experimental study of trachoma has been handicapped by the lack of a laboratory method of culturing the aetiological agent. Until recently top priority was given to the problem of laboratory cultivation since upon its accomplishment depended a whole series of investigations which would pave the way to a complete knowledge of the nature of the trachoma agent including its relationship with the agent of inclusion conjunctivitis.

T'ang et al.\textsuperscript{1} succeeded for the first time in growing the trachoma virus in the yolksac of developing chick embryos and since then isolations\textsuperscript{2-15} have been reported from many parts of the world. With the availability of the virus in quantity investigations continue to extend the range of immunological tests in the laboratory diagnosis of trachoma. Diagnostic antigens have been prepared in several laboratories using different purification procedures and employed in a variety of immunological tests. The current study is aimed at the preparation of diagnostic antigen and the evaluation of complement fixation test and skin test as compared to the microscopic detection of inclusion bodies.
The Trachoma Pilot Project sponsored by the Indian Council of Medical Research in collaboration with the World Health Organisation have recently made a preliminary epidemiological survey in India on the basis of clinical examinations. It was felt that such investigation would become comprehensive and precise if an appropriate diagnostic laboratory test is adopted. The need for a laboratory test is very real in the incipient or proctochomatous stage of the disease, cases in which the underlying trachoma is obscured by overwhelming secondary bacterial infections, cases of low activity in which conjunctival or corneal changes are minimal and in borderline cases occurring in the heavily trachomatized population of India.

Laboratory diagnosis of trachoma is usually based on the demonstration of Halberstaedter-Prowazek type of inclusion bodies in conjunctival cells by microscopic examination. Unfortunately, diagnostic value of inclusion bodies is limited, first by the fact that microscopically identical inclusions occur in inclusion conjunctivitis and second by the fact that in trachoma of low activity inclusions may be so few in number as to escape detection in the usual single-slide examination.

It has been observed by a number of investigators that sera from trachoma patients contain antibodies which fix complement in the presence of psittacosis, ornithosis and lymphogranuloma antigens. It is now generally recognised that trachoma virus shares with psittacosis-lympho-
granuloma group of viruses a common group antigen and that definite slight general immunity reaction occurs in trachoma. Group specific antigens prepared by the method of phenolization and boiling are commonly used in complement fixation tests for the laboratory diagnosis of diseases caused by psittacosis-lymphogranuloma group of viruses. Such antigen obtained from the trachoma virus is not adequately sensitive to detect the usual low titre antibodies in clinically established cases of trachoma. Investigators in U.S. Naval Medical Research Unit No.2 have employed purified elementary body antigen from trachoma virus in complement fixation tests for laboratory diagnosis of trachoma and they claim that the antigen is specific, reacting only with the sera from trachoma patients and sensitive, detecting even low level antibodies.

According to the Report of Medical Research Council for the year 1957-58, the injection of trachoma virus gives a positive skin reaction in trachoma patients but not in clinically normal subjects. The skin test using trachoma antigen seems to offer a prospective method of laboratory diagnosis in trachoma as in case of lymphogranuloma venereum.

The present investigation undertaken in 1958 was primarily directed to the laboratory cultivation of trachoma virus and subsequently extended to the purification of antigen for use in appropriate laboratory
diagnostic test. While working in the All India Institute of Medical Sciences, New Delhi, three strains of trachoma virus were isolated and serially passaged in embryonated eggs, employing the yolksac route of inoculation.

While at the U.S. Naval Medical Research Unit No.2, crude antigens prepared from well adapted strains of trachoma virus were purified by the method of fluorocarbon emulsification and differential centrifugation. Along with the purification of antigen the role of lecithin and other components bearing on the antigenic composition of the virus studied. Purification experiments using varying amounts of fluorocarbon were designed with a view to determine the optimum amount which would remove anticomplementary factors and non-viral yolksac elements without impairing the viral antigen. The antigen prepared by the optimal amount of fluorocarbon was tested with a variety of sera to establish its purity and specific antigenic reactivity and standardized for use in diagnostic tests.

At the Trachoma Research Centre, Aligarh the optimally purified elementary body antigen was used for diagnostic complement fixation and skin tests. Conjunctival scrapings from all the persons tested by CFT and skin test were examined microscopically for Halberstaedter-Prowazek type of inclusion bodies.

Results of the three tests are compared in relation to the clinical diagnosis made by the ophthalmologist. Evaluation of a test is based on specificity and
sensitivity. Of the three diagnostic tests evaluated in the present work complement fixation method employing the optimally purified elementary body antigen proves most reliable.