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WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

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WHO EXPERT COMMITTEE ON HEPATITIS

Second Report

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in a marked shift in the usual age and sex distribution of cases in these countries when outbreaks associated with this vehicle for infection recently occurred. Predominance of cases among adults has also been observed when serum hepatitis constitutes a significant proportion of total cases in the community. In such situations preventive measures are available and can be applied if the mode of transmission is identified.

Though it might reasonably be expected that mortality data would be more accurate than morbidity data, this is not true with present reporting practices. Deaths from viral hepatitis may be included under a variety of other classifications, and jaundice due to other etiologies may be inaccurately coded as due to viral hepatitis. It is usually not possible to evaluate the accuracy of coding those deaths ascribed to acute and subacute necrosis of the liver and to other possible sequelae of viral hepatitis. A comparison of morbidity/mortality ratios, in various countries, however, may be useful as a guide to under- and over-reporting of both types of data.

EPIDEMIOLOGY OF VIRAL HEPATITIS

Information on the period of infectivity of patients with serum hepatitis and infectious hepatitis has been obtained from epidemiological studies and volunteer experiments.

Infectivity of infectious hepatitis

Faecal filtrates from infectious hepatitis patients have been shown to transmit the disease when administered to volunteers by the oral route. Serum is infective by both the oral and parenteral routes. Transmission, therefore, can occur from person to person by the faecal-oral route, and also as a result of transfer of blood by any procedure which breaks the skin or mucous membranes. Blood and serum are infective during approximately the same period as faeces, and virus is excreted in the faeces of individuals infected by the parenteral route. In studies of experimentally infected subjects viraemia and faecal excretion of the agent have been demonstrated during the pre-icteric stage and have ceased by about three weeks after onset of jaundice. Studies of urine and nasopharyngeal washings are limited and inconclusive.

In one series of experimental transmission studies with a virus having an average incubation period of 40 days, the agent was demonstrated to be in the faeces of icteric patients from about 16 days before until between one and eight days after the onset of jaundice. Individuals with anicteric infections are believed to excrete the virus for a comparable period of time. Virus excretion between eight and 18 days after onset of jaundice was not studied.

Attempts to transmit the disease with faecal pools collected during convalescence, 19 to 33 days after onset of jaundice, were unsuccessful. In another study of two children with chronic anicteric hepatitis, infective faeces were obtained five and 15 months respectively after the diagnosis was made, but it is not known whether they were infective throughout the whole of these periods.

Infectivity of serum hepatitis

Experimental transmission of serum hepatitis has been produced by blood taken as long as 89 days before the onset of symptoms and four to eight days after jaundice has occurred. It is known from follow-up studies of blood transfusions that the blood of some donors may be continuously or intermittently infective for many years. Blood and blood fractions containing serum hepatitis virus have produced disease experimentally only when given parenterally. Faeces, urine and nasopharyngeal washings administered orally have not been shown to be infective.

Environmental factors

Available data indicate that most cases of infectious hepatitis are due to person-to-person transmission, the effectiveness of which appears to be related to the closeness of contact. Persons living in the same household as a patient are at the greatest risk. Spread between families and in communities usually occurs as a result of activities which provide for close contact between preschool and school-age children. In most studies the lower socio-economic groups have a higher prevalence of infectious hepatitis in childhood, presumably as a result of greater crowding, poorer sanitation and less adequate personal hygiene.

The recognition of water- and food-borne epidemics of hepatitis has resulted in an increased awareness of the potential importance of this mode of spread. In Delhi, India, an estimated 29 000 cases occurred in 1955-56, as a result of contamination of the municipal water supply. Elsewhere, four epidemics spread by milk have been reported ; in two of these, contaminated water used to wash dairy equipment was responsible. Ingestion of raw shellfish from polluted waters is known to have caused three epidemics. The incriminated clams and oysters were widely distributed and cases occurred in different parts of the country, thus making it difficult to recognize the common vehicle of infection. Contamination during preparation has resulted in transmission by other foods, including custard, sandwiches, orange juice, salads and cooked meat.

Mechanical transfer of the virus from faeces to food or eating-utensils by flies and cockroaches has been suggested, but there is no evidence that this mode of transmission has any significance.

The parenteral transmission of serum hepatitis or infectious hepatitis usually occurs in one of three ways: (1) through therapeutic administration of blood and unsterilized blood products or, rarely, by transplantation of human tissue; (2) by use of a contaminated instrument which has broken the skin of two persons, the first of whom was viraemic; and (3) through accidental cuts or scratches.

The risk of transmitting viral hepatitis by the use of blood and untreated blood products may be summarized as follows:

Whole blood. The frequency of viral hepatitis from the transfusion of whole blood ranges between 0.09% and 4.1% (usually less than 1%). The larger the number of units administered, the greater the risk of transmitting the disease. In some areas it has been observed that blood from donors receiving payment has been responsible for a higher frequency of hepatitis than that obtained from voluntary donors.

Plasma. Untreated pooled human plasma carries a higher risk than whole blood and the risk increases with the size of the pool. The attack rates in several studies ranged from 0.12% to 12.2%.

Fibrinogen. Fibrinogen cannot be sterilized without loss of its biological properties. The assessment of the risk it involves is difficult because it is generally given with whole blood. The attack rate, which in one instance was 17%, appears to be related to the size of the pool from which the fibrinogen is prepared.

Antihaemophilic globulin. The risk with antihaemophilic globulin is probably similar to that from fibrinogen.

Thrombin. Thrombin prepared by the ethanol or by the ether method may transmit serum hepatitis.

There have been no cases of hepatitis attributable to gamma-globulin prepared by the cold ethanol method of Cohn, and only one doubtful case attributable to gamma-globulin prepared by the cold ether method. Available evidence indicates that ammonium sulfate precipitation and ethacridine (Rivanol) precipitation are also safe methods of preparation, but specific follow-up studies have not been reported. Other products prepared from pooled plasma can be sterilized by heat. Stable plasma protein solution, albumen, fibrin foam and plasminogen appropriately treated are safe.

Viral hepatitis has been transmitted parenterally by needles, tubing, bottles and syringes used for intravenous, intramuscular, subcutaneous, and intradermal injections; needles and syringes used for venepuncture, lancets used for scarification and capillary puncture; dental equipment; tattooing needles; and improvised equipment used by narcotic addicts.

Accidental inoculation of medical, nursing and laboratory personnel dealing with patients or handling human blood is well recognized. In

Sweden a curious form of accidental infection has been recently described in cross-country runners with scratches on their limbs.

Transplacental transmission of serum hepatitis has been suggested on the basis of very limited observations which require confirmation.

No evidence for transmission of either infectious or serum hepatitis by haemophagous arthropods has been produced.

Host factors

Infection may or may not result from exposure. When infection takes place it may be apparent or inapparent. Estimates of the ratio of apparent to inapparent cases vary widely depending on the laboratory tests used and the population surveyed. Natural resistance may play some role in determining whether infection will occur but there are no methods for evaluating it. Acquired immunity plays a demonstrable role in protection against the homologous agent. The duration of protection is unknown, but from epidemiological observations it is presumed to be life-long in most instances.

Second attacks of icteric disease, both presumably due to infectious hepatitis virus, have been observed in up to 5% of patients. The interval between such episodes varies from several months to many years. It has also been found recently that inapparent infections may occur in persons who some months later develop icteric disease. A number of hypotheses can be brought forward to explain second attacks (for example, infection with antigenically distinct strains, or the recrudescence of a chronic process), but more information on the etiology and immunology of the infection is required before the mechanism of second attacks can be clearly understood.

Epidemics of both infectious and serum hepatitis which were associated with common vehicles of infection and in which large populations were apparently exposed to the same degree of infection have brought to light some factors other than acquired immunity which influence the attack rates. During water-borne epidemics of infectious hepatitis in Sweden, India and the United States of America attack rates increased with age up to 25 years. In Delhi the incidence in pregnant women was significantly higher than in non-pregnant women of the same age-group.

In the late 1930s and early 1940s many people were inoculated with yellow fever vaccine containing human serum contaminated with serum hepatitis virus. It was found that the attack rates of viral hepatitis varied with age and with ethnic group, the older age-groups and white races having the higher attack rates.

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WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
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THE USE OF HUMAN IMMUNOGLOBULIN

Report of a WHO Expert Committee

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antibody on coupling antigen to adsorbent, ensuring adequate elution of the antibody (especially of that fraction with the highest binding capacity for antigen), and ensuring that the recovered antibody is unchanged with respect to its biological properties, neutralizing ability and rate of elimination from the body. This was considered an important area for research, and when antigenic determinants associated with neutralizing ability ultimately become available, it will be possible to extend the methods to the specific purification of the therapeutically effective antibodies to each infectious agent.

5. PROCUREMENT OF PLASMA FOR IMMUNOGLOBULIN PREPARATION

The difficulty of obtaining sufficient plasma to meet the demand for human immunoglobulin is a major problem. On the other hand, the problems of supply are not insuperable, since sufficient quantities of human immunoglobulin preparations to meet national requirements are being produced in some countries. Methods of obtaining plasma may be considered separately for the two categories of human immunoglobulin.

5.1 Human normal immunoglobulin

In a number of countries, this is already produced, in amounts sufficient to meet the present national need, from time-expired plasma provided by blood transfusion units, from plasma specially collected for the purpose, or from human placentas. However, if the need for immunoglobulin increases, as, for example, if its value in preventing serum hepatitis is firmly established, then difficulties will be encountered in meeting the demand. Among possible methods of increasing plasma supplies the Committee considered that the most promising was the removal of a quantity of plasma routinely from all blood donations. This can be accomplished by keeping the blood in a bag to the neck of which is attached a small side-bag sufficient to take about 75-100 ml of plasma, which can be squeezed into it when the blood cells have settled. It has been estimated that in the USA this procedure could increase the annual supply of plasma for immunoglobulin preparation from 300 000 litres to approximately 750 000 litres, an increase of about 150 %. The Committee also noted that the use of human placentas is insufficiently exploited in many countries.

5.2 Human specific immunoglobulin preparations

A number of methods of obtaining plasma rich in specific antibodies are practised.

Certain antibodies, such as those of mumps, rubella, measles and variola, can be obtained from convalescent patients, but the quantities obtainable in this way clearly cannot be large. Supplies of convalescent plasma depend on the co-operation of general medical practitioners and of hospitals, institutions and recruit camps, and the difficulties are mainly those of organization.

The supply of plasma from persons who have been specially immunized has been sucessfully practised on a relatively small scale for a number of immunoglobulin preparations (e.g., in the case of tetanus, pertussis and rabies), but considerable difficulties are anticipated in achieving large-scale supplies. Good supplies of human tetanus antitoxin have been obtained in some countries by the use of volunteers from the armed forces and from industrial groups, such as miners, and steel workers, amongst whom active immunization is common.

Where routine immunization of pregnant women is employed, the use of human placentas may constitute a source of specific immunoglobulin, for example, tetanus antitoxin.

Work is being done to find other methods of concentrating specific human antibodies. Thus the possibility, which was discussed earlier, of extracting antibody from plasma by means of antigens attached to supporting particles, might eventually prove of value. The potency of such preparations would need to be assessed to ensure that protective properties remained.

It was noted that subjects in some geographical zones, notably West Africa, have very high plasma concentrations of γG or γM immunoglobulins. There are indications that these high levels may not be entirely due to the intensity of exposure to infectious agents and that differences in the degree of the antibody response may be involved. Interesting information might therefore be obtained from studies of the antibody-producing capacity of different human populations.

It is possible that human immunoglobulin from certain geographical areas might prove to be rich in particular antibodies for which a demand exists elsewhere. Information is therefore required concerning the amount of various antibodies to be found in immunoglobulin preparations from different parts of the world.

The screening of normal time-expired plasma has been used in certain countries to allow the selection of samples rich in particular antibodies.

Increased plasma yields have been obtained by means of plasmapheresis and this procedure may become increasingly important, particularly in the case of specifically immunized donors.

There is evidence that the type of vaccine, the immunization schedule and the time interval between giving an antigen booster and taking plasma are of considerable importance in obtaining maximum antibody titres.

Investigation of these points is necessary so that optimum antibody yields can be obtained from immunized donors, although the immunization and bleeding schedules to be used may have to be established separately for each immunizing antigen.

It may be necessary, in order to obtain sufficient immunized donors, to resort to payment; this has proved successful in some areas in the case of both tetanus and mumps immunoglobulin.

An interesting possibility is to try to obtain from the general population volunteers who will allow themselves to be immunized and then contribute plasma, rather in the same manner as ordinary blood donors. By suitable appeals, it might well be possible to obtain large numbers of such volunteers.

Finally, it is important that attention should be given to ways of decreasing the demand for immunoglobulin. Thus, in the case of tetanus, diphtheria, measles, pertussis, etc., active immunization campaigns should have an appreciable effect. The development of methods whereby blood could be freed from hepatitis viruses would eliminate any need to give immunoglobulin with blood transfusions. In the meantime, it may be desirable to take blood from patients routinely before operations so that their own blood can be available for transfusion if necessary, particularly since it is now possible to preserve blood from individuals by long-term, low-temperature storage.

6. CLINICAL APPLICATION OF HUMAN IMMUNOGLOBULINS

6.1 Introduction

Human immunoglobulin is used in both prophylaxis and treatment to provide passive immunity by virtue of the specific antibodies it contains. Although it has been used in a wide variety of conditions, good evidence of its effectiveness exists only for a small number of diseases, and there is a great need for studies from which the value of human immunoglobulin can be estimated. Animal antisera have been used in a number of conditions for many years and there is no doubt that they should be replaced by the corresponding human immunoglobulins. This is recommended with the aim both of eliminating hypersensitivity reactions to animal sera and of prolonging the time during which passively injected antibody circulates in the blood. The various diseases considered in this section are mainly those in which passive immunization is either of undoubted use or should carefully be considered in the light of available evidence. In addition, a number of instances are discussed in which recent work suggests that a role for passive immunization may develop in the future.

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Nineteenth Report

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General Consideration

Human immunoglobulin preparations [1] have been in use for many years and there can be no doubt of their efficacy for the prevention and treatment of various infectious diseases. These preparations contain immunoglobulins mainly of the IgG class.

Recent advances in epidemiology, virology and immunology have given a better understanding of the natural history of a number of diseases.

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[1] A WHO Expert Committee on the Use of Human Immunoglobulin recommended that the term "immunoglobulin" should be used instead of previously used names, such as "gamma globulin" and "immune serum globulin" (*Wld Hlth Org. techn. Rep. Ser.*, 1966, 327).

Moreover, techniques for assaying antibodies against a number of viruses have been developed and corresponding international standards and reference preparations are now available so that the results of such assays can be expressed in uniform terms. These developments, together with recent progress in immunochemistry, have provided a better basis for the production, control and clinical use of human immunoglobulin.

Human immunoglobulin is a safe product and rarely causes side-effects when given intramuscularly. The requirements are generally the same, whether the preparations are made from plasma or from placental material, as both preparations should have the same properties with regard to efficacy and safety.

Recently, a WHO Expert Committee on the Use of Human Immunoglobulin [1] gave special attention to the clinical use and efficacy of immunoglobulin preparations in a number of diseases. The Committee recognized two distinct categories of human immunoglobulin: (*a*) human normal immunoglobulin, which is a preparation made from pooled human plasma from a large number of randomly selected donors; and (*b*) human immunoglobulin specific for a particular antibody, which is a preparation made from pooled human plasma obtained from convalescent patients, immunized donors or selected antibody-rich plasma sources. The requirements formulated below relate to both these categories.

The requirements for human normal immunoglobulin have been formulated to ensure the effectiveness of these preparations in different diseases, including those for which the estimation of protective antibodies is not yet practicable, such as infectious hepatitis and some bacterial diseases. Potency requirements specific for each of the various antibodies that normal immunoglobulin may contain are not given, but some requirements are specified to ensure that no substantial loss of antibodies present in the original plasma pool has occurred during fractionation. The requirements for human immunoglobulin specific for a particular antibody have been formulated to ensure that a definite amount of the particular antibody is present. Thus, it is required that the potency is expressed in terms of international units for each of three different specific human immunoglobulin preparations available at present, namely those containing tetanus antitoxin, measles antibody and vaccinia antibody. When other specific human immunoglobulin preparations have been developed and their efficacy has been demonstrated, it will be necessary to include such preparations in future revisions of these requirements.

The present requirements relate to three methods of production using: (*a*) alcohol, (*b*) ether, and (*c*) ammonium sulfate. All three methods provide products that are safe in respect of the transmission of homologous serum hepatitis, but that contain predominantly only those antibodies that

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, 327.

are present in immunoglobulins belonging to the IgG class.[1] New procedures for the preparation of immunoglobulin are being developed, but until they have been shown to yield a safe product, especially with regard to the transmission of hepatitis, adequate requirements for them cannot be formulated. National control authorities are advised to take this into consideration before such products are released for general use.

Immunoglobulin preparations generally available at present cannot be used for intravenous administration because of the risk of untoward reactions in some recipients. It is desirable, however, to have immunoglobulin preparations suitable for intravenous use, since they would permit the rapid attainment of high titres of circulating antibody and allow the painless injection of relatively large volumes. During the last few years some promising results with preparations suitable for intravenous administration have been reported. Existing information, however, is not sufficient to enable adequate requirements for such preparations to be formulated. The present requirements have therefore been formulated to cover preparations intended for intramuscular administration only.

No precise tests have been included in the requirements to give an indication of the degree of degradation of immunoglobulin during preparation and storage. Such tests would be useful to ensure that the product after administration to man is maintained at an effective level and for an adequate time. Some indications might be obtained for example by ultracentrifugal and immuno-electrophoretical studies, combined with blood-level duration studies. The national control authority should take into consideration all available information on this question in deciding on the tests to be prescribed for particular types of immunoglobulin preparations and methods of manufacture (see Part B, section 1).

In view of the continued research and the rapid advances in the field of human immunoglobulin, it is foreseen that the present requirements are likely to need revision within a few years.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements so that, if a health administration so desires, these parts as they appear may be used as definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning human immunoglobulin, it is recommended that a clause be included that would permit modifications of manufacturing requirements on the condition that it be demonstrated, to the satisfaction of the national control authority, that such modified

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, 327.

requirements ensure that the safety and potency of human immunoglobulin are at least equal to those provided by the requirements formulated below. It is desirable that the World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory", as used in these requirements, always refer to the country in which the human immunoglobulin is manufactured.

Part A. Manufacturing Requirements

1. Definitions

1.1 *International name and proper name*

The international name applicable to all preparations shall be *Immunoglobulinum humanum*. Preparations of *Immunoglobulinum humanum* may be divided into two groups. One is designated *Immunoglobulinum humanum normale* and the other includes the preparations designated as follows:

Immunoglobulinum humanum antitetanicum,
Immunoglobulinum humanum antimorbillicum,
Immunoglobulinum humanum antivacciniosum.

The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to preparations of human immunoglobulin that satisfy the requirements formulated below.

1.2 *Descriptive definition*

Immunoglobulinum humanum is a preparation of immunoglobulin of human origin intended generally for the prevention and treatment of infectious diseases. It is prepared either from human plasma or from material containing human plasma proteins.

Immunoglobulinum humanum normale is prepared from pooled material from at least 1000 human donors.

Immunoglobulinum humanum anti(x) [1] contains specified amounts of antibodies against designated viral or bacterial agents or bacterial toxins and therefore may be prepared from pooled plasma from a limited number of donors who are either convalescent patients or immunized donors.

[1] (x) is to be replaced in each particular case by the appropriate name given in Part A, section 1.1.