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# THE USE OF HUMAN IMMUNOGLOBULIN

## Report of a WHO Expert Committee

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WORLD HEALTH ORGANIZATION

GENEVA

1966

## WHO EXPERT COMMITTEE ON THE USE OF HUMAN IMMUNOGLOBULIN

Geneva, 7-13 September 1955

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# THE USE OF HUMAN IMMUNOGLOBULIN

## Report of a WHO Expert Committee

The WHO Expert Committee on the Use of Human Immunoglobulin met in Geneva, from 7 to 13 September 1965. Dr P. Dorolle, Deputy Director General, in opening the meeting on behalf of the Director General, said that WHO had convened the present Expert Committee to discuss problems associated with the increased use of human immunoglobulin, to review the present situation and to estimate future needs, to advise on areas in which research is needed, and to make recommendations concerning current usage. Dr J. J. van Loghem was elected Chairman, Dr A. Hässig Vice-Chairman and Dr S. Krugman Rapporteur.

### 1. INTRODUCTION

Human gamma globulin preparations have been used for the prevention of various infectious diseases for a period of about 20 years, after studies of the original gamma globulin preparations had revealed the presence of a wide variety of antibodies. By 1945, the efficacy of such preparations for the modification and prevention of measles and infectious hepatitis was established. In addition, gamma globulin preparations containing high levels of particular antibodies have been produced by fractionation of plasma obtained from specially immunized individuals.

During the past two decades, the use of human gamma globulin has increased substantially. It has been employed in three principal ways:

- (1) for the prophylaxis of certain viral diseases, e.g., measles, infectious hepatitis and rubella;
- (2) for the prophylaxis and therapy of tetanus and other diseases in order to overcome the disadvantages of the heterologous serum preparations;
- (3) for the treatment of antibody deficiency syndromes to prevent recurrent bacterial infections.

The future needs and uses of human gamma globulin may be modified significantly. The development and use of safe and effective vaccines

will decrease the need for passive protection. The results of recent studies on the value of gamma globulin for prophylaxis and treatment of a number of diseases may have a profound effect on current practice, in some fields increasing, in others decreasing, the demand for gamma globulin.

## 2. TERMINOLOGY

The Committee recognized the lack of agreement in terminology used in various countries. Substantial differences exist between the commonly used name and the terms recommended in the nomenclature for human immunoglobulins published by WHO.<sup>1</sup> Since these terms have been widely accepted, the Committee considered that the following designations should be used.

The generic name will be "*human immunoglobulin*" (*immunoglobulinum humanum*).<sup>2</sup>

Preparations obtained by use of random pools of human plasma may be referred to as "*human normal immunoglobulin*" (*immunoglobulinum humanum normale*).<sup>2</sup> This designation corresponds to previously used names such as "gamma globulin", "normal gamma globulin", or "immune serum globulin".

Preparations containing specified amounts of particular antibodies should be designated "*human immunoglobulin anti-*" (*immunoglobulinum humanum anti-*)<sup>2</sup> followed by the name of the appropriate antigen (x). This includes preparations previously designated as "hyperimmune" or "convalescent gamma globulin". Human immunoglobulin anti-(x) can be prepared by using plasma obtained from convalescent patients or immunized donors or by selecting some antibody-rich plasma source. This group may be referred to in general terms as "human specific immunoglobulin".

## 3. HETEROGENEITY OF ANTIBODIES AND IMMUNOGLOBULINS

The study of the immunochemistry of antibodies formed in man has shown that they may be composed of any or all of three classes of serum proteins designated as  $\gamma G$ ,  $\gamma M$  and  $\gamma A$  immunoglobulins.<sup>1</sup> A fourth

<sup>1</sup> *Bull. Wld Hlth Org.*, 1964, 30, 447-450.

<sup>2</sup> This terminology is open to modification if preparations containing particular immunoglobulin fractions become available, e.g., human immunoglobulin ( $\gamma M$ ) anti-(x).

class of immunoglobulin, termed  $\gamma D$ , has recently been described but as yet antibodies are not known to be associated with it. None of these classes of immunoglobulins represents a homogeneous population of identical molecules, but each consists of a large population of molecules which differ in physical, chemical, immunological and other biological properties. While antibodies of known specificity are found in these three classes of immunoglobulins, there are substantial quantities of immunoglobulins in normal serum which are not known to possess antibody activity. On intensive immunization, especially in animals, antibody can be formed in quantities that constitute a significant proportion of one or another of the immunoglobulin classes.

The  $\gamma G$  immunoglobulin, which is present in largest amount in serum (about 1 g/100 ml) has been shown to be made up of 4 polypeptide chains (two identical heavy chains and two identical light chains), into which it can be split by reduction of some of its disulfide bonds with mercaptoethanol. The light and heavy chains may readily be separated by chromatography in acid solution. Recombination of the heavy and light chains of  $\gamma G$  immunoglobulin has been shown to take place with restoration of physical and chemical properties. Immunoglobulins of  $\gamma A$  and  $\gamma M$  classes can also be split into subunits by reduction of disulfide bonds, but the breakdown products have not been so well studied.

Many independent parameters of heterogeneity of the  $\gamma G$ ,  $\gamma M$  and  $\gamma A$  immunoglobulins have been recognized. All three classes show a broad spectrum of mobility in immunoelectrophoresis. Individual  $\gamma G$  molecules differ from one another in the mobility of their light chains when subjected to starch-gel electrophoresis in mercaptoethanol-urea at alkaline pH; 10 distinct light-chain bands have been recognized.  $\gamma G$  myeloma proteins obtained from the serum of patients with Waldenström's macroglobulinaemia have been shown to be much more homogeneous, having electrophoretic mobilities in a narrow range and generally exhibiting a single sharp band comprising their light chains; they may thus represent products of a single clone of neoplastic cells.

Not only do the Bence-Jones proteins of each patient differ in mobility but they also differ in antigenic specificity. Rabbit antisera to individual Bence-Jones proteins may be classified in two broad groups, designated K and L, having different antigenic determinants. The myeloma protein and the Bence-Jones protein from a given individual are always type K or type L but never both. Light chains of K and L types occur in all three classes of immunoglobulin. Some evidence for a third antigenic type of light chain has also been found. The Bence-Jones proteins of patients with myeloma and macroglobulinaemia have been shown to be of the same type as the light chains of the myeloma protein or macroglobulin of the same patient and appear chemically to be light chains or dimers thereof.

Most purified antibodies in any of the three major classes of immunoglobulins may also occur as immunoglobulins with  $\kappa$  or  $\lambda$  light chains. Some antibodies, however, notably the  $\gamma$ M cold agglutinins of blood group anti-I specificity, have been found only with  $\kappa$  light chains.

Using antisera prepared against individual myeloma globulins and suitably absorbed, four types of heavy chains have been recognized in the  $\gamma$ G immunoglobulins. These  $\gamma$ G immunoglobulin subgroups appear to differ in certain biological properties, such as their ability to attach themselves to guinea-pig skin to give reverse passive cutaneous anaphylaxis.

An additional parameter of heterogeneity is the presence of certain allotypic antigenic determinants on individual molecules of some immunoglobulins. Among these are the Gm and Inv factors.<sup>1</sup> The occurrence of these determinants follows classical Mendelian genetics. Gm is associated with the heavy chain of  $\gamma$ G globulin, while Inv may occur on light chains of all classes of immunoglobulins. Antibodies and myeloma globulins may possess only one of the Gm or Inv factors present in whole  $\gamma$ G immunoglobulins from the same individual, or indeed may lack any known Gm or Inv factor. Some correlation between the Gm group and the heavy-chain subgroup offers the possibility of simplifying knowledge of these parameters.

Recent studies of the amino-acid sequence on several Bence-Jones proteins of type K have shown them to consist of a region of the same amino-acid sequence and another region of variable sequence—i.e., differing from one Bence-Jones protein to another. Evidence also exists that the heavy chains consist of a constant and a variable region. The nature of the attachment of the variable to the constant region is not known, and the possibility of an eight-chain structure for  $\gamma$ G immunoglobulin exists. Cases have been reported in which part of the Bence-Jones protein in urine consists only of the variable portion.

Purified human antibodies to four antigens produced in one individual have been found to show considerable variation in their amino-acid compositions, suggesting that, like the myeloma, they also represent products of selected populations of immunoglobulin-synthesizing cells.

Digestion with papain splits  $\gamma$ G immunoglobulin and  $\gamma$ G antibodies into three fragments, two of which (the Fab fragments) possess antibody combining sites and the third (Fc) is inactive. With pepsin, a bivalent antibody fragment is formed with digestion of about one-third of the molecule into small fragments; this bivalent fragment can then be split into monovalent fragments by reduction of a single disulfide bond. The fragments possessing the antibody-combining sites consist of the light chain and the variable portion of the heavy chain (Fd). With some anti-

<sup>1</sup> Notation for genetic factors of human immunoglobulins (1965) *Bull. Wld Hlth Org.*, 33, 721.

bodies, the bivalent fragment is almost as potent as the intact  $\gamma$ G antibody, but some decrease in potency accompanies reduction to monovalent fragments.

In addition to the parameters considered thus far, antibody populations also exhibit heterogeneity with respect to the specificity of their combining sites. The antibody even to a single well-defined antigen is heterogeneous in that it can be shown to contain antibodies of different specificities. Studies with antipolysaccharide antibodies, such as anti-dextran, formed in man have shown that heterogeneous populations of antibody molecules are formed, even against what appears to be a single antigenic determinant.

Individual antisera produced in different animals may contain antibodies to all or only to some of the antigenic determinants on the antigen, and the proportion of antibodies formed to each determinant may vary from animal to animal. Antibody to perhaps only one of the different determinants may be associated with a given biological property, e.g., have antitoxic properties, etc. The isolation of single antigenic determinants of individual proteins and the elucidation of their structure is an important area of current research which is being actively pursued.

It is probable that it will be possible to associate other biological properties of antibodies with structural parameters of heterogeneity and additional parameters may well be discovered. The immunoglobulin- or antibody-synthesizing cells recognized by existing methods are thus seen to consist of thousands of different cells, each synthesizing at least one kind of immunoglobulin molecule. The number of classes of immunoglobulin molecule, as well as the kinds of antibody molecule that a given cell may synthesize and the specificity and heterogeneity distribution of the antibody formed, have not yet been clearly established.

#### 4. PRODUCTION PROCEDURES FOR THE PREPARATION OF IMMUNOGLOBULIN CONCENTRATES

Three procedures have found wide-scale application for the production of immunoglobulin concentrates. These are the cold-ethanol procedures of the late E. J. Cohn and his associates, the ether technique of Kekwick and Mackay, and ammonium sulfate precipitation. In immunoglobulin preparations containing 16 g of protein per 100 ml, these procedures provide concentrations of 10-20 times those of the antibodies of the original plasma. They are highly reproducible and provide products free from known virus contamination. Control of pyrogenicity of the product is easy with the cold-ethanol and ether techniques, but requires much greater attention when salting-out techniques are used.

The ether process has been little used and has been found difficult to expand owing to the problems of maintaining a closed system in the handling of large batches and the dangers of working with ether. The cold-ethanol technique has had by far the greatest application. It is imperfect, however, since some alterations of the plasma proteins are produced and it is commonly held to be too costly.

New, less expensive and gentler techniques have been studied, such as adsorption of other plasma proteins on DEAE cellulose or DEAE Sephadex at pH 7, leaving the immunoglobulins in a solution from which they can readily be concentrated. This procedure has not been used widely and the immunoglobulin concentrates so prepared have yet to be proved free from infective virus.

Ensuring that the final preparation is free from the etiological agent of serum hepatitis is perhaps the most serious barrier to application of new developments in fractionation procedures; possible means of overcoming this are as follows:

(1) Culture of the etiological agents would permit direct *in vitro* assessment of possible risks of new processes.

(2) Progress on a small scale can be made by the use of selected groups of donors who can be considered probably free from carrying the hepatitis virus.

(3) An acceptable virucidal technique could be applied to immunoglobulin prepared by new procedures. The most generally accepted method, that of heating for ten hours at 60°C, cannot be applied to immunoglobulin solutions without increasing their viscosity to an extent that would entail loss of storage life. Another technique, application of ultraviolet light and betapropiolactone, as described by LoGrippo, does not destroy the antibodies in whole plasma and might well be used successfully with immunoglobulin solutions.

(4) It may be possible to take advantage of circumstances in which new methods of fractionation are the only ones available and where the advantages of using them outweigh the dangers of transmitting hepatitis. Thus, for example, the use of antimalaria immunoglobulin prepared by column fractionation is being studied and careful follow-up of the recipients is providing valuable information on the safety of this method of fractionation.

Another area demanding improvement of processing technique concerns the separation and concentration of  $\gamma$ M immunoglobulins, in which certain antibacterial antibodies are found. The present cold-ethanol and ether methods provide products that are essentially  $\gamma$ G immunoglobulin (the aggregates seen are apparently polymers of normal  $\gamma$ G molecules). There is a need for preparations that would be effective against infections caused by *Pseudomonas aeruginosa*, *Escherichia coli* and

other bacteria; to obtain such preparations it may be necessary to develop new methods that will concentrate immunoglobulins other than those found in the preparations at present available.

Immunoglobulin solutions prepared from human plasma by cold-ethanol or ether processes may be stored at 4°C for three to six years with little evidence of change. However, proteolytic fragmentation in some immunoglobulin preparations, predominantly those of placental origin, has been observed during storage at 4°C and this may affect both antibody content and clinical efficacy. Further studies on these observations are recommended and the possibility of adding effective and safe chemical stabilizers should be investigated. The absence of a significant increase in 3.5 S molecules during storage of immunoglobulins for one month at 37°C may be used as a stability test for these solutions.

The usual concentration of human immunoglobulin in final preparations is 16 g/100 ml and in the subsequent sections of this report this concentration is implied wherever dosage is referred to.

#### 4.1 Preparations for intravenous use

It is desirable to have some immunoglobulin preparations suitable for intravenous use, since this permits the rapid attainment of a high titre of circulating antibodies and allows the injection of relatively large volumes painlessly. The solutions usually available are not suitable for intravenous use since they provoke reactions in a proportion of patients, particularly those with antibody-deficiency syndromes. Following the intravenous injection of immunoglobulins, most patients become temporarily tolerant to subsequent intravenous doses of immunoglobulin. After such desensitization, the refractory phase may last from 2 to 5 days depending on the dosage used. Preparations digested by proteolytic enzymes do not have this effect. However, desensitization may be hazardous and precautions should be taken when patients are given injections of such preparations.

The untoward reactions to intravenous administration are believed to be due to the anticomplementary activity of aggregated immunoglobulin molecules. These may be removed by ultracentrifugation, which is impracticable on a large scale, or by enzymatic degradation, which has been shown to reduce the half-life by over 50%.

A more promising method is incubation of immunoglobulin at pH 4 and 37°C. This has yielded preparations free of anticomplementary activity that are well tolerated intravenously and that possess a half-life only moderately shorter than that of the untreated material. Some batches so treated may not be freed entirely from anticomplementary activity and addition of trace amounts of pepsin may be needed. The process yields a stable preparation with little or no 5.5 S components.

The final preparation contains 5-10% of a 10-11 S component which is devoid of anticomplementary activity and is equal in antibody potency to the original material.<sup>1</sup> Before preparations treated at pH 4 are released for clinical use, it is imperative to test them for absence of anticomplementary activity. Since some preparations have shown reappearance of anticomplementary material, or cryoprecipitation on standing, the shelf-life of these materials requires further study.

In Switzerland, over 250 patients have received therapy intravenously with immunoglobulins treated at pH 4 and more than 20 patients with antibody-deficiency syndromes have been treated for two years without any untoward reactions. Two other groups have obtained similar results.

#### 4.2 Isolation of antibodies

Human normal immunoglobulin contains antibodies effective in the prophylaxis and therapy of several diseases and its use in any single disease results in wastage of the other antibodies contained in the injected dose. Since human immunoglobulin is in short supply, it is obvious that separation of each of these antibodies would permit optimal utilization of existing supplies and the recovery of antibodies present in human plasma in concentrations too low to be otherwise useful. Possible methods of isolating specific antibodies were considered, the basis of which is the specific adsorption of the antibody with antigen and the elution of the antibody from the antigen-antibody complex. Such methods have been used to purify a wide variety of antibodies from antisera of various animal species. When insoluble antigens that retain their binding capacity for antibody can be obtained by coupling the antigen to insoluble adsorbents by covalent linkages or by attaching groups that render the antigen insoluble, antibody may be recovered which is essentially free of contamination with antigen. Insoluble adsorbents are also advantageous in that they permit concentration and recovery of antibodies present in low concentrations. Antibodies may be adsorbed on columns of these insoluble antigens or may be adsorbed batchwise. Such methods offer the most promise for separating and isolating individual antibodies. Methods of elution involving the use of specific haptens of low molecular weight or a reduction in pH have been used. To date, these techniques have not been applied to the therapeutically useful antibodies present in human immunoglobulins, but experience already acquired on rabbit and horse antibodies to protein antigens and on human anti-dextran and human anti-A and anti-B should prove helpful. Major difficulties to be surmounted involve: the preparation of adequate quantities of purified antigens, viruses, toxins, etc., avoidance of loss of binding capacity for

<sup>1</sup> Barandum, S., Kistler, P., Jeunet, F. & Isliker, H. (1962) *Vox Sang. (Basel)*, 7, 157-174.

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 successfully 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  $\gamma$ G or  $\gamma$ M 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.

The source of the human immunoglobulin used varies in different diseases. In some instances, where contact with the disease is widespread, pooled normal plasma has proved to be a satisfactory source of protective antibodies, e.g., measles, infectious hepatitis, rubella. It has sometimes become possible to titrate the appropriate antibodies in batches of normal pooled plasma, thereby enabling suitable lots to be designated for use in the corresponding disease, e.g., measles. However, in the majority of instances, in order to obtain an adequate concentration of protective antibodies, it is necessary to use plasma either from convalescent patients, as in the case of mumps, or, more commonly, from volunteer donors who have deliberately been immunized against the specific disease.

The dose of human immunoglobulin which is recommended for use in the various conditions is based on the published data, but it is emphasized that the information available is often limited and that there is a great need for investigations to decide optimal prophylactic and therapeutic dosage. In studies with human immunoglobulins, it is important that doses should be specified in terms of IU if they are available, so that the effectiveness of therapy in different areas can be properly evaluated. Whenever possible, the doses are given in terms of the IU, but when an international unitage has not been established they are given in terms of a solution of human immunoglobulin containing 16 g/100 ml, unless otherwise stated.

Human immunoglobulin is given intramuscularly in most cases, and, apart from slight discomfort at the site of injection in occasional patients, side-effects are very rare. However, the preparations usually available are not suitable for intravenous use because they provoke reactions in a proportion of recipients, particularly those with antibody deficiency syndromes. The reactions may take the form of discomfort, flushing of the face, or circulatory collapse with loss of consciousness. Work on this problem is referred to elsewhere in this report (section 4.1, page 9), and it seems likely that preparations suitable for intravenous injection will become available.

## 6.2 Tetanus

Human tetanus antitoxin is needed for the replacement of heterologous antitoxin used in prophylaxis and therapy. This may, however, be difficult to achieve owing to the large amounts that are used throughout the world and because it is prepared from the plasma of deliberately immunized donors. The extension of active immunization and the use of chemoprophylaxis in place of antitoxin in patients with clean wounds treated soon after injury may well decrease the requirement for antitoxin considerably. Nevertheless, adequate supplies of antitoxin are required

for the treatment of patients with tetanus and for prophylaxis in patients who are judged to require antitoxin but are known to be hypersensitive to animal serum.

#### *Prophylaxis*

The titre of passive antibodies that is protective has been variously estimated to be between 0.01 and 0.2 IU per ml, but most authors accept a figure of 0.01-0.05 IU per ml. Such concentrations can be maintained in man for 3-4 weeks after the injection of between 250 and 500 IU of homologous antitoxin, and the routine prophylactic dose probably should lie in this range for all age groups. Simultaneous active and passive immunization is now widely practised when antitoxin is given to non-immunized patients, and it is probable that the smaller dose of human antitoxin causes less interference with the development of active immunity when heterologous antitoxin is used.

#### *Therapy*

Comparative studies have shown that there is no need to give doses larger than 20 000 IU of horse serum antitoxin in the treatment of tetanus, and this dose is widely used. There is no sound evidence that homologous antitoxin is more effective and the dose used should probably be the same as that of heterologous antitoxin. Antitoxin is often given intravenously in treating tetanus in order to achieve a high titre of circulating antibody as soon as possible, but the human antitetanus immunoglobulin available at the present time cannot be injected in this way.

### **6.3 Diphtheria**

Heterologous antitoxin is used for prophylaxis and treatment of diphtheria and is generally believed to be of considerable value. The widespread use of active immunization has led to effective control of diphtheria in many parts of the world and passive immunization is required only rarely when small outbreaks occur. The best yields of human antitoxin will undoubtedly be obtained from the plasma of deliberately immunized donors.

#### *Prophylaxis*

The prophylactic dose of heterologous antitoxin commonly recommended for children is 500-1000 IU. There appears to be no information about homologous antitoxin. It might be argued that the dose should be similar to that of the heterologous preparation, since it is possible that the plasma concentration in the first few days following the injection of the antitoxin is of particular importance in diphtheria, because of the short incubation period.

### *Treatment*

The dose of heterologous antitoxin used in treatment is commonly of the order of 200 to 1000 IU per kg body weight. Similar doses of human antitoxin will probably be required, since there is no evidence that it is more effective in therapy than heterologous antitoxin.

### **6.4 Smallpox and vaccinia**

There are two possible sources of human anti-vaccinia immunoglobulin, plasma obtained from patients convalescent from smallpox, and plasma obtained some weeks after vaccination against smallpox. In the former case, the antigen is a natural circulating variola virus; in the latter it is vaccinia virus. The antibody titre in convalescent plasma from smallpox patients has been reported to be approximately 50 times higher than in serum from vaccinated persons. There are no major detectable differences between the antigenic composition of vaccinia and variola strains so that plasma from either source can be used.

### *Prophylaxis*

*Post-vaccinal encephalitis.* In most parts of the world routine prophylaxis is unnecessary because of the low incidence of this complication. However, in some countries, where it is more common, especially in adults, the use of human anti-vaccinia immunoglobulin has been shown to be of value. An effective dose is 2 ml given intramuscularly simultaneously with the vaccination.<sup>1</sup>

*Eczema vaccinatum.* Smallpox vaccination of patients suffering from eczema is generally contraindicated. However, vaccination may be indicated for travel to areas where smallpox is highly endemic. Under these circumstances, a 2-ml dose<sup>1</sup> should be given simultaneously with vaccination.

*Variola.* It has been shown in a controlled study that 5-10 ml (dependent on age) of a 12% solution of anti-vaccinia human immunoglobulin given during the incubation period significantly reduces the incidence of smallpox. This treatment is not a substitute for vaccination either before or immediately after contact; it is of most value in unvaccinated contacts who are discovered late in the presumptive incubation period. The availability of effective chemoprophylactic agents will reduce the need for immunoglobulin prophylaxis.

<sup>1</sup> The International Standard for Antismallpox Serum is a freeze-dried preparation of pooled human post-variola serum, and is dispensed in ampoules each containing 1000 IU. In the international collaborative assay made to establish this international standard, a number of materials were assayed, and of these a preparation of post-vaccination human immunoglobulin assayed at 1000 IU per ml. In other studies in two countries a number of such preparations assayed at between 900 and 1800 IU per ml. These solutions contained 16 g per 100 ml.

### *Treatment*

Human anti-vaccinia immunoglobulin has been reported to be effective for the treatment of the following serious complications of vaccinia: generalized vaccinia, eczema vaccinatum, and vaccinia gangrenosa. Accidental introduction of virus into the eye would be an indication for treatment because of the risk of severe scarring and potential blindness.

## **6.5 Measles**

The availability and use of safe and effective measles-virus vaccines will significantly decrease the need for human immunoglobulin for passive protection against measles. Human normal immunoglobulin is commonly used but since it is possible to specify the content of anti-measles antibody, dosage should ideally be expressed in terms of IU.<sup>1</sup> Prophylaxis is indicated for presumably susceptible infants and children who are exposed to measles. Since the attack rate following exposure may range between 20% and 90%, live attenuated measles-virus vaccine should be given simultaneously at a separate site for active immunization of those children who may have escaped infection.

### *Prophylaxis*

The dose of human normal immunoglobulin is 0.04 ml per kg of body weight, to be given as soon as possible after exposure, preferably within 5 days. This dose will usually modify rather than prevent measles; the attenuated disease will be followed by permanent immunity. A larger "preventive" dose (0.2 ml/kg) is indicated for infants who have an acute or chronic severe debilitating disease. Simultaneous inoculation of live measles vaccine is indicated only for children who receive the smaller dose of immunoglobulin (0.04 ml/kg); larger doses may suppress the immunogenic effect of the live vaccine.

### *Treatment*

Human immunoglobulin preparations have not been proved to be of value for the treatment of measles.

## **6.6 Rubella**

Rubella is a mild and relatively benign disease of childhood. Even in adults, complications are rare. On the other hand, the occurrence of rubella infection during the first trimester of pregnancy may be followed by a high incidence of congenital malformations, stillbirths and abortions.

<sup>1</sup> In one country, an investigation of ten human normal immunoglobulin preparations (16 g/100 ml) showed that the content of anti-measles antibody varied from 70 to 300 IU per ml.

The epidemic of rubella in the USA in 1964 was followed by the birth of many thousands of infants with congenital malformations. Many mothers of affected infants had subclinical rubella. These infants exhibited one or more of the following manifestations: (1) intrauterine growth retardation; (2) thrombocytopenic purpura; (3) eye lesions, such as cataract and glaucoma; (4) deafness; (5) congenital heart disease; (6) brain lesions associated with microcephaly, hydrocephalus or meningoencephalitis; (7) hepatitis, and (8) bone lesions.

The cultivation of rubella virus in tissue culture in 1962 provided the techniques needed to study the natural history of rubella and to evaluate the efficacy of human anti-rubella immunoglobulin. The studies of the natural history of rubella have revealed the following significant findings:

(1) Virus was isolated from the throat of rubella patients as early as 7 days before and as late as 21 days after onset of rash. Therefore, susceptible contacts may be infected before the disease is recognized clinically.

(2) Viraemia was present 7 days before the onset of rash but it was not observed after the second day of rash. Therefore, a pregnant woman with rubella may infect her foetus before the disease is recognized clinically. Thus the use of any prophylactic agent after appearance of the rash would probably be of no value.

(3) Rubella-neutralizing antibody was usually detectable by the third day after onset of rash; it probably persists for a lifetime thereafter. The presence of neutralizing antibody in the serum was indicative of immunity. In a recent study of 54 children who did not have detectable serum antibody, 46 (85%) contracted rubella after intensive exposure. In contrast, in a group of 37 children with detectable antibody none of the children contracted the disease after a comparable exposure. In no instance did an immune person show evidence of viraemia. Therefore, an immune pregnant woman probably has adequate protection against the transmission of rubella to her foetus *in utero*.

### *Prophylaxis*

The use of human immunoglobulin for prevention of rubella has been a controversial subject for many years. The results of at least seven separate controlled studies reported in the literature since 1949 are completely contradictory—ranging from no protection at all to significant protection against clinical disease.

Careful studies<sup>1</sup> have recently been made in which experimental

<sup>1</sup> Green, R. H. et al. (1965) *J. dis. Child.*, **110**, 348.

conditions simulated the type of exposure to which a pregnant women could be subjected. Children with no pre-existing antibody were exposed to cases of rubella. They were given human normal immunoglobulin, 0.24-0.40 ml per kg of body weight; it was inoculated within 24 hours of the development of rash in the contagious cases. The attack rate in the treated children (85%) did not differ significantly from that in children acting as controls (92%).

During another recent study of an epidemic of rubella in Alaska,<sup>2</sup> a significant reduction in the rubella infection rate was observed among children receiving a large dose of human immunoglobulin (0.55 ml per kg of body weight). The attack rate of rubella infection was 44% in the immunoglobulin group as compared with 86% in the uninoculated control group. Under the conditions of this trial, many of the subjects received immunoglobulin before exposure.

It is important to differentiate between the rationale for the use of immunoglobulin for the prophylaxis of rubella as compared with measles and infectious hepatitis. The aim in measles and in hepatitis is to modify or prevent a potentially severe disease in an exposed person. On the other hand, the aim in rubella is to prevent infection of the foetus. Foetal infection probably results from viraemia in the mother. There was no significant effect of immunoglobulin on the incidence of rubella infection and viraemia under conditions that simulated the type of exposure to which a pregnant woman may be subjected.

There are many well documented reports of congenital malformations in infants whose mothers received a 20-ml dose of human normal immunoglobulin shortly after exposure to rubella. However, at the present time there are no controlled studies available to answer the crucial question—does human immunoglobulin reduce the risk of congenital malformations, stillbirths and abortions? In the case of a pregnant woman for whom a therapeutic abortion may be considered if infection occurred, the immunoglobulin should be withheld as it may mask the clinical disease. On the other hand, if a therapeutic abortion would not be carried out, the use of human immunoglobulin should be recommended because of the possibility that the risk of congenital rubella may be decreased. At the present time the use of human immunoglobulin for the prevention of rubella can no longer be recommended as a routine procedure; the indications for its use must be individualized.

Because of the importance of the problem, the development of vaccines for active immunization, particularly of young females, is essential. In addition, it is important to provide facilities for measuring rubella antibodies in serum to determine if exposed women have been infected.

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<sup>2</sup> Brody, J. A., Sever, J. L. & Schiffi, G. M. (1965) *New Eng. J. Med.*, **272**, 127-129.

### 6.7 Infectious hepatitis

The Committee fully endorses the recommendations given in the second report of the WHO Expert Committee on Hepatitis.<sup>1</sup> These recommendations are reproduced here in full:

“The efficacy of gamma-globulin in the prevention of clinical infectious hepatitis has been established by studies in many parts of the world. The priorities for the administration of gamma-globulin will vary from country to country. The aim of any programme should be, within the limits of the amounts available, to protect as many of those at high risk as possible and also those, such as pregnant women, in whom the illness may be more serious than usual.

“Gamma-globulin in a dose of 0.02 ml to 0.04 ml of a 16% concentration per kg of body-weight will effectively prevent hepatitis with jaundice in the usual short-term type of contact exposure—for example, in a household. Gamma-globulin is of value if given at any stage of the incubation period, but the earlier the better. It is of no value in treatment. A dose of 0.06-0.12 ml per kg will give a longer period of protection, perhaps up to five or six months. Consequently where supplies allow, the larger dosage may be indicated for potentially susceptible persons travelling to areas where the incidence is thought to be high. In these circumstances it is probable that not more than two doses are required at an interval of five to six months, since under conditions of high exposure inapparent infection during passive protection should give active immunity.”

### 6.8 Serum hepatitis

The incidence of hepatitis with jaundice following blood transfusion has been reported to be about 1-3 cases per thousand donations (“units”) of blood used; occasionally it has been as high as 15 cases per thousand. The risk is increased in high-incidence areas and in situations in which donors are not carefully selected. The overall mortality rate from post-transfusion hepatitis has been reported to range between 0.9% and 27.5% in hospitalized cases of hepatitis.

A recent report<sup>2</sup> indicated that the incidence of post-transfusion hepatitis with jaundice was decreased significantly by the use of human normal immunoglobulin. Patients were given 10 ml within one week following transfusion and a similar dose one month later. The incidence of hepatitis with jaundice was 1.55% or 3.2 cases per thousand “units” of blood in the immunoglobulin treated group, as compared to 4.04% or 9.2 cases per thousand “units” in the control group. The incidence

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1964, **285**, 22.

<sup>2</sup> Mirick, G. S., Ward, R. & McCollum, R.W. (1965) *New Eng. J. Med.*, **273**, 59-65.

of anicteric hepatitis was the same in both groups: 6.2% or 13 cases per thousand "units". This study confirms earlier work, employing essentially the same dosage-time schedule, in which the incidence of icteric hepatitis was reduced from 8.9% in controls to 1.3% in those receiving immunoglobulin.

Supplies of human immunoglobulin are quite inadequate to meet a demand for the routine use of human immunoglobulin for the prevention of post-transfusion serum hepatitis. Nevertheless, use of human immunoglobulin would probably be justified for certain "high-risk" patients. The following factors should be considered in deciding which patients are at high risk; the presence of chronic illness, particularly in the elderly, the giving of multiple transfusions, and pregnancy. Special consideration should also be given to the local incidence of serum hepatitis. The dosage that has been used is 10 ml within 7 to 10 days after transfusion, followed by a second dose one month later. Any recommendation that human immunoglobulin be used prophylactically presupposes, however, that all methods of minimizing the risk of post-transfusion hepatitis mentioned in this report and elsewhere have been implemented.

### 6.8 Varicella

Epidemiological studies have indicated that human immunoglobulin in large doses will not prevent but may attenuate varicella. Prophylaxis is not indicated for normal children. The use of immunoglobulin should be limited to the following high-risk cases: (1) newborn and premature infants; (2) debilitated infants under 6 months of age; (3) children with blood dyscrasias, especially those on corticosteroid therapy, and (4) certain susceptible adults (severe, potentially fatal varicella pneumonia is most common in adults and very rare in children).

The recommended dose of human normal immunoglobulin is 0.4-1.0 ml per kg of body weight, given as soon as possible after exposure.

### 6.9 Mumps

Human immunoglobulin for use in mumps is prepared from plasma obtained from artificially immunized donors. The value of this preparation for the prevention of mumps is disputed. Mumps in children is a relatively mild disease and prophylactic measures are not indicated. On the other hand, large doses of immunoglobulin prepared from convalescent patients (20 ml intramuscularly) were shown to be valuable for the prevention of orchitis in adolescent and adult males if given shortly after onset of the disease.

### 6.10 Poliomyelitis

The availability of safe and effective poliomyelitis vaccines has eliminated the need for immunoglobulin for the prevention of poliomyelitis.

### 6.11 Rabies

There is evidence that passive immunization, using not less than 40 IU of antirabies serum prepared in animals, when given simultaneously with active immunization, is effective in preventing the development of rabies in persons exposed to a high risk of infection. In some countries, plasma for the preparation of human antirabies immunoglobulin has recently been obtained from actively immunized donors. There is, however, need of more information on the protective dose of human antirabies immunoglobulin, and on possible interference with active immunization.

### 6.12 Antibody-deficiency syndromes (hypo- and agammaglobulinaemia)

These syndromes are characterized by an impaired ability to synthesize antibody. Most cases are characterized by a deficiency or lack of immunoglobulin components in the blood.

In the congenital, acquired and symptomatic (associated with a given disease) forms of antibody-deficiency syndrome, it is possible to protect the patient from acute episodes of bacterial infection. The recommended dose of human normal immunoglobulin is 1.2-1.8 ml/kg of body weight given intramuscularly at least once each month. During acute infections, the same dosage of immunoglobulin should accompany appropriate antibiotic therapy. To avoid the pain and discomfort of injecting such large doses intramuscularly it is important to develop methods of ensuring that preparations of human immunoglobulin are safe when given intravenously.

Patients maintained with monthly injections of human normal immunoglobulin do not seem to be protected against chronic infections of the respiratory and gastrointestinal tract. In certain forms of hypogammaglobulinaemia (familial lymphopenia, Swiss-type agammaglobulinaemia) substitution therapy with immunoglobulin is ineffective.

If agammaglobulinaemia or hypogammaglobulinaemia is due to increased catabolism or urinary loss of immunoglobulins (e.g., protein-losing enteropathy) there is no antibody deficiency and therefore no need for treatment with immunoglobulin.

### 6.13 Pertussis

Pertussis immunoglobulin is prepared from the plasma of donors immunized with pertussis vaccines. Relatively large amounts of antibody may be obtained by plasmaphoresis. The efficacy of the available preparations for the prophylaxis and treatment of pertussis is doubtful. However, in view of the relatively high mortality rate of the disease in infants less than 6 months old, antipertussis immunoglobulin may be used in this age group.

### 6.14 Other bacterial infections

Human normal immunoglobulin has been shown to contain antibodies against various micro-organisms, and immunoglobulin has been observed to have a favourable effect on infections in animal experiments. Human experience is limited chiefly to case reports of patients who received combined therapy. In these cases, antimicrobial therapy seemed to be ineffective until immunoglobulin was included in the therapeutic regimen. In burned patients, combined therapy with antimicrobials and large doses of immunoglobulin appeared to reduce the incidence of septicæmia and late mortality generally observed following treatment with antibiotics alone. Further controlled studies are needed.

### 6.15 Protozoal and parasitic diseases

There is good evidence that immunoglobulin prepared from the plasma of adults and from the cord blood of babies in West Africa has antiparasitic effects on *Plasmodium falciparum* in West and East Africa. There is need for similar studies to be undertaken in other protozoal and parasitic diseases.

The amount of immunoglobulin required to produce a favourable response in malaria is large and for this reason it is unlikely to find general application in the prophylaxis and treatment of malaria at the present time. However, its possible use in the treatment or modification of infections with strains of malarial parasites that are resistant to the available antimalarial drugs should be borne in mind.

### 6.16 Haemolytic disease of the newborn

The Committee noted the encouraging preliminary results of studies in several countries, in which immunoglobulin prepared from plasma containing incomplete antibody to the Rh(D) antigen is being used with the aim of preventing the occurrence of haemolytic disease of the new-

born. In general, the methods employ immunoglobulin prepared from the plasma of specially immunized volunteers. This source of incomplete anti-D antibodies is given 24 hours after delivery to Rh-negative women who have appreciable numbers of foetal red blood cells in their circulation. The injected antibody promotes the disappearance of the foetal cells and apparently depresses the antibody response of the mother to the D antigen; it is hoped that this will prevent the development of haemolytic disease in subsequent pregnancies.

#### **6.17 Influenza and other respiratory virus infections**

Human immunoglobulin has been used for the prophylaxis and treatment of influenza and other respiratory virus infections. The results have not been consistent. In various well controlled epidemiological surveys in military recruits there was no evidence of any beneficial effect. However, the Committee was informed of initial favourable studies of the effects of anti-influenza immunoglobulin for the prevention and treatment of influenza. The Committee recommended further well controlled studies of this problem. At present, the administration of immunoglobulin for the prevention and treatment of respiratory virus infections is not recommended.

#### **6.18 Gas gangrene**

Vaccines suitable for use in man are not yet available and there seems to be no immediate possibility of preparing human gas-gangrene antitoxin. The need for this preparation is probably small owing to the use of antibiotics, but the possibility of satisfying such a demand should be borne in mind.

#### **6.19 Botulism**

A definite demand exists for small amounts of antitoxin for use in prophylaxis and therapy. Since vaccines suitable for use in volunteer donors have been described, efforts to prepare the corresponding immunoglobulins should be encouraged.

#### **6.20 Snake-bite**

There are few detoxified venoms suitable for the immunization of man, but the current world-wide interest in the production of antivenom encourages the Committee to suggest that consideration should be given to the production of human antivenoms.

### 6.21 Staphylococcal diseases

Human immunoglobulin prepared from volunteers immunized with staphylococcus toxoid is prepared in some countries. In view of the doubt concerning the value of this product the Committee did not feel able to recommend its general production, but considers that further studies be made in order to assess its usefulness.

### 6.22 Other potential uses of immunoglobulin

This report deals chiefly with the prophylactic and therapeutic effects of immunoglobulins in the light of their function as specific antibodies. However, immunoglobulins may have other properties, as indicated by observations such as the desensitization phenomenon which may occur after intravenous injection in humans and suppression of anaphylaxis in animals. These and other observations suggest that these proteins have a biological function at the cell surface which should be more thoroughly explored. At present, however, the scientific basis for this concept is not yet sufficiently understood.

## 7. REQUIREMENTS FOR HUMAN IMMUNOGLOBULINS

The Committee agreed with the view expressed in the seventeenth report of the WHO Expert Committee on Biological Standardization<sup>1</sup> that there is a need to formulate international requirements for human immunoglobulin preparations. The Committee considered the following points to be important:

### 7.1 Pooling of plasma

In the present state of knowledge, the value of human immunoglobulin preparations depends on the presence of various representative antibodies found in the plasma of normal individuals. It is important, therefore, that pools of plasma should be prepared from a large number of donations (of the order of 1000 or more), when normal pooled immunoglobulin is to be prepared. Human specific immunoglobulin can be prepared from pools derived from a smaller number of donors.

### 7.2 Safety tests

Animal tests to establish that human immunoglobulin preparations are non-toxic are satisfactory, and human immunoglobulins are among

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1964, **293**, 23.

the safest biological products. Additional safety tests are needed, however, for preparations intended for intravenous use, and it is recommended that investigations to develop such tests should be started.

### 7.3 Potency tests

In the case of many diseases for which specific immunoglobulin preparations are used, there is a great need for information about the dose that should be given and this is an important area for further research. Determination of therapeutic and prophylactic dosage should, wherever possible, be expressed in international units. This information should be used to formulate requirements for the antibody potency of each preparation. It should also be noted that it may eventually become necessary to determine the content of IgM or IgA antibodies in immunoglobulin preparations, if these are shown to be important in particular diseases. Human normal immunoglobulin should also have its content of antibodies measured, again in terms of international units. It is likely that differences will be found in the content of various antibodies in preparations from different countries and a knowledge of these variations is important to ensure that human normal immunoglobulin does always contain antibodies appropriate to the disease for which it is prescribed.

### 7.4 Stability tests

The quality control of immunoglobulin preparations is important and they should be stable in respect of physicochemical and immunochemical composition and biological activity. Methods proved to give decisive information about the quality and stability of immunoglobulin preparations should be included in control requirements. Useful methods of evaluating stability are: change in composition after heating, estimation of aggregates, and estimation of the formation of 3.5 S molecules after storage at 37°C for one month. When antibody potency can be measured directly, it would be particularly desirable to use the rate of elimination from the blood as a measure of quality. It would be of interest to relate changes in the composition of immunoglobulin preparations during storage to changes that may occur in the content of specific antibodies.

### 7.5 Expiry date

Immunoglobulins stored at approximately 4°C are reported to be very stable. However, storage conditions after release are often far from ideal, and this must be considered when recommending an expiry period.

## 8. RECOMMENDATIONS

1. There is a great need to develop reliable procedures for sterilizing blood and blood products without altering their biological properties. The availability of such techniques would greatly facilitate the development and application of new fractionation procedures, which at present are hampered by the necessity of evaluating products for the absence of hepatitis virus by tests in man.

2. As an immediate practical method to be used where there are shortages of plasma for fractionation into human normal immunoglobulin, it is strongly recommended that a quantity of plasma, e.g., 75-100 ml, be taken routinely from all blood transfusions. One method of accomplishing this without compromising the sterility of the blood, is to collect the donated blood in a plastic bag at the neck of which is a small side-bag sufficient to hold 75-100 ml of plasma, which can be squeezed into it when the blood cells have settled.

3. Further work is needed to develop methods for ensuring that some human immunoglobulin preparations are made suitable for intravenous injection, e.g., tetanus antitoxin intended for therapeutic use. The methods discussed in this report are promising.

4. Adequate methods should be developed for the separation and concentration of immunoglobulins, e.g.,  $\gamma$ M antibodies, which are largely absent in the present immunoglobulin preparations.

5. In order to obtain the highest yields of specific antibodies, studies are needed for all the diseases for which human specific immunoglobulin is prepared, to determine the most suitable vaccines, immunization procedures and bleeding schedules.

6. Efforts should be made to determine the antibody content of human immunoglobulins prepared in different parts of the world, and means should be made available by which representative samples could be tested. The information so obtained may lead to the possibility of exchange between different countries when preparations from some areas prove to be rich in antibodies deficient in others.

7. It is possible that variations might be found in the ability of different populations to respond to different antigens. If this proved to be so, it might be practicable to use this difference for procuring antibody-rich plasma; studies of this possibility should be made.

8. To minimize the risks of serum hepatitis and conserve stocks of immunoglobulin, individual countries are strongly urged to introduce procedures of delayed autotransfusion, whereby plasma obtained by plasmapheresis, as well as whole blood, can be taken from patients pre-opera-

tively and kept for use when the patient is hospitalized for operation. In addition, the possible applications of the technique of storing blood from individuals at low temperatures for long periods should be explored.

9. Fundamental studies should be encouraged on the specific separation and concentration of individual antibodies from pools of normal human plasma.

10. Although the low incidence of reactions to human immunoglobulins injected intramuscularly suggests that at this time there is no significant clinical problem due to antibodies to allotypic specificities of immunoglobulins such as the Gm and Inv group, additional data on the incidence and levels of allotypic antibodies following injections of immunoglobulin solutions should be accumulated. This is especially important since it is probable that supplies of immunoglobulin for intravenous use will increase, and if reactions due to anti-allotype antibodies do occur, they may be more severe after intravenous injection of immunoglobulin.

11. Further studies are required on the value of human immunoglobulin in many conditions, and this is a very important and wide field for further research. Particularly important are studies on the treatment of bacterial infections, and also deserving particular mention is the need for studies in the field of protozoal and parasitic diseases.

12. Because of the need for more information on the usefulness of human immunoglobulin for modifying post-transfusion serum hepatitis, it is recommended that WHO encourage appropriate controlled studies in areas with high incidence of serum hepatitis so that the necessary data may become available.

13. In diseases where immunoglobulins *are* known to be effective, there is need for studies to determine the optimal prophylactic and therapeutic doses. The importance of specifying dosage in terms of international units is emphasized, so that studies in different regions can be properly evaluated.

14. There is need for studies to assess the possible protective efficacy of antibodies found in the immunoglobulin classes other than  $\gamma$ G. Since  $\gamma$ M and  $\gamma$ A immunoglobulins are not transferred across the placenta to any appreciable degree, the newborn infant may be regarded as hypogammaglobulinaemic in respect to these two immunoglobulin classes. Studies should be made, therefore, of the efficacy of  $\gamma$ M and  $\gamma$ A immunoglobulin preparations in infections in newborn infants. Studies should also be undertaken to evaluate the antibody response of populations with high concentrations of  $\gamma$ G immunoglobulin (e.g., in patients with malaria) and  $\gamma$ M immunoglobulin (e.g., in patients with trypanosomiasis), as well

as the antibody response of agammaglobulinaemic children with normal  $\gamma$ A and  $\gamma$ M immunoglobulins.

15. More knowledge is needed on the toxin- and virus-neutralizing capacities of enzymatically produced fragments of immunoglobulins to determine whether they have a potential clinical application.

16. Data are required on the methods by which degradation of immunoglobulins on storage can be prevented.

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