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# IMMUNOLOGICAL ASPECTS OF HUMAN REPRODUCTION

Report of a WHO Scientific Group

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WHO SCIENTIFIC GROUP ON THE IMMUNOLOGICAL ASPECTS  
OF HUMAN REPRODUCTION

Geneva, 4-9 October 1965

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# IMMUNOLOGICAL ASPECTS OF HUMAN REPRODUCTION

## Report of a WHO Scientific Group

A WHO Scientific Group on Immunological Aspects of Human Reproduction met in Geneva from 4 to 9 October 1965. The meeting was opened by Dr L. Kaprio, Director, Public Health Services, who welcomed the members on behalf of the Director-General.

Professor F. W. Rogers Brambell was elected Chairman and Dr O. E. Vjasov Vice-Chairman. Dr R. G. Edwards and Dr G. A. Voisin agreed to act as Rapporteurs.

### 1. INTRODUCTION

1.1 Within the last ten years there have been significant advances in knowledge of protein chemistry. These are mainly concerned with methods of purification and analysis, biosynthesis and chemical synthesis, and the controlled splitting of protein molecules. The application of such methods will certainly help to unravel some of the outstanding problems in many fields, including those concerned with immunological processes. These advances are of increasing importance to research in reproduction, and to an understanding of reproductive processes. The subject of immunology is complex, and largely made so by the great number of substances involved and a lack of knowledge of their detailed structure.

1.2 A fundamental problem in all immunological investigations concerns the purification of the antigens. Ion exchange chromatography on the modified celluloses and gel filtration are two comparatively recent techniques that have been particularly useful. There are several well-known physicochemical criteria for judging the homogeneity of such preparations. These include free and starch-gel electrophoresis, ultracentrifugation, and solubility studies. It will be noted that these are techniques that detect impurities or resolve a protein mixture into its constituents: they do not give a positive indication of purity. Many different tests are required before a high degree of purity can be claimed for any preparation. These should include not only physicochemical tests but immunological techniques, such as double diffusion or immuno-electrophoresis and, where possible, specific assays of biological activity.

1.3 It must be stressed that even in highly purified preparations of protein the biologically active sites do not necessarily act as antigenic determinants. Sometimes the antibody inhibits the biological activity of the protein but not by a direct action on the antigenic site. In other cases, there is no change in biological activity when the antigen reacts with the antibody, and it can happen that specific antibodies even enhance certain enzymic activities. Wherever possible it is advisable to perform immunological and biological assays in parallel.

1.4 Some topics not discussed at length might well form subjects of discussion for future WHO Scientific Groups, e.g., incompatibility for the Rhesus blood group antigens in relation to haemolytic disease of the newborn, and immunological tolerance. Other topics have been deliberately excluded from this report since at present little experimental work has been reported on them, e.g., the immunological activities of ova and of the foetus, and anaphylaxis in the newborn caused by bovine milk (cot-death).

### Terminology

1.5 The Group noted that certain terms relating to immunology and reproduction need defining more clearly and suggested that these questions should receive the attention of the appropriate international bodies. For the purposes of this report, the following definitions were adopted for different categories of antigens and antibodies :

Auto-antigen :	A substance that is antigenic in the individual from whom it was obtained.
Iso-antigen :	A substance that is not antigenic in the individual from whom it was obtained but is antigenic in other members of the same species.
Hetero-antigen :	A substance that is antigenic in a species other than that from which it was obtained.
Auto-antibody :	An antibody that reacts with an antigen belonging to the same individual.
Iso-antibody :	An antibody that reacts with an antigen belonging to the same species, but not with the antigens of the individual in whom it was found.
Hetero-antibody :	An antibody that reacts with an antigen belonging to a species other than that in which it was found.

The terms "fertile", "infertile" and "sterile" were taken as being applicable to both the male and the female, i.e., an aspermatic male is con-

sidered infertile or sterile ; a female who is unable to conceive after insemination, or who cannot give birth to living young, is considered infertile or sterile. If a male can inseminate a female he is considered fertile.

## 2. IMMUNOLOGY OF HUMAN GONADOTROPHINS

### Human chorionic gonadotrophin

2.1 This hormone appears to be a glycoprotein, the carbohydrate component being essential for its biological activity. Although considerable progress has been made in its purification it has not been obtained absolutely pure as judged by immunological criteria. Some commercially available samples of human chorionic gonadotrophin (HCG) and other preparations claimed to be highly purified may give antibodies reacting in agar gel not only with HCG but with human luteinizing hormone (HLH) and human follicle stimulating hormone (HFSH). It is likely that the substance giving rise to the anti-HFSH antibody occurs as a contaminant. There are methods described that are claimed to separate these gonadotrophins and the application of such methods to the purification of HCG is awaited with interest.

2.2 It is probable that several related proteins are measured in the immunological tests at present used for HCG since there is ample evidence to show that there is some dissociation of biological and serological activities. The production of HCG in the early stages of pregnancy, however, is so great that these impurities do not affect the accuracy of immunological pregnancy tests, the reliability of which is well documented.

2.3 Further work is required to identify chemically the active molecule and to produce, if possible, an antibody directed against the biologically active sites. For the routine diagnosis of pregnancy the haemagglutination methods, or the extremely rapid modification using latex particles, are unlikely to be replaced by other immunological techniques for some time. Complement fixation is also reliable, particularly for estimations of HCG in serum, and is preferred by some workers.

2.4 Greater sensitivity may be achieved by radio-immunoassay using HCG labelled with  $^{131}\text{I}$ . This may be important in special clinical problems, such as the follow-up of cases of chorioepithelioma.

### Human luteinizing hormone (HLH or HICSH)

2.5 The HLH of the pituitary has biological properties similar to HCG. Compared with other gonadotrophins it has been obtained in a high degree of purity, the only biologically active contaminant being a trace of thyro-

trophic hormone. There have been conflicting reports concerning its immunological properties. The majority of antisera to HCG cross-react with purified HLH in double diffusion or haemagglutination tests but it remains to be proved that the reactions are concerned with the biologically active components. It is interesting, and perhaps relevant, that a chemical difference exists between the two hormones, i.e., that HCG contains considerably more sialic acids (*N*-acetylneuraminic and *N*-glycolylneuraminic acids) than HLH. Cross-reactions between pituitary HLH preparations of different species have been observed. The significance of these observations remains to be elucidated.

2.6 The relationship between the chemical nature of HLH as it occurs in the pituitary and in the urine or blood has not been established. Urinary HLH appears to react immunologically with antisera to HCG so that the excretion of HLH may be assessed by the haemagglutination-inhibition method using antisera to HCG or HLH. There are several claims that the mid-cycle peak in HLH associated with ovulation may be recognized by use of such a method. This deserves extensive investigation since, if confirmed, the method would afford a valuable indication of the time of ovulation. The available immunological tests are sufficiently sensitive to detect HLH in a reasonably small quantity of urine within one or two hours. If the sensitivity could be increased still further, so that concentration of the urine was unnecessary, it would be an improvement that would permit of the method being applied to serum as well. The <sup>131</sup>I-immunoassay would probably be sufficiently sensitive but, in its present form, it takes 4-5 days.

#### **Human follicle stimulating hormone (HFSH)**

2.7 This hormone has proved difficult to purify. It appears to be a glycoprotein and as purification progresses it becomes less stable. It is possible that a protein such as albumin protects it during the early stages of purification. The removal of the last traces of albumin and of HLH is very difficult but by the use of refined techniques, including ion-exchange chromatography, starch-gel electrophoresis and solvent partitioning, a preparation with a high specific biological activity has been obtained.

2.8 Even so, it still seems that a specific immunological assay for HFSH is at present not available. Antisera to the pituitary hormone cross-react with the HFSH component of urinary gonadotrophins and not with HCG or with pituitary gonadotrophins of other species, except perhaps the monkey. However, the purest HLH available, in which it is not possible to show any HFSH activity, inhibits the agglutination of red cells coated with HFSH by antiserum to HFSH. This suggests that the antigenic groups may be common in both the HFSH and HLH molecules.

**Biological effects of anti-gonadotrophins**

2.9 There is general agreement that antisera to human gonadotrophins strongly inhibit the effect of human gonadotrophins injected into experimental animals. It is not yet clear whether the inhibition is specifically directed against the hormone used as antigen. There have been a few studies on the effect of these antibodies on endogenous hormones but so far no serious attempt has been made to produce immunity to gonadotrophic hormones.

2.10 There is no indication that treatment of infertile women or men with human gonadotrophins (HFSH and HCG) leads to the formation of antibodies, although no studies have been reported in which the hormones were administered with adjuvants. Gonadotrophins of other species however, e.g., pregnant mare serum gonadotrophin, may give rise to antibodies, but the preparations commonly used have been so crude that the suspected antibodies may have arisen in response to proteins other than the hormone.

**Gonadotrophin-inhibiting material (GIM)**

2.11 There have been a number of interesting observations suggesting that GIM occurs in the urine of children of both sexes and also in the urine of women at various stages of the normal menstrual cycle. The material is stable to heat and acid. Its action is directed against HCG but not against HFSH or oestrogens. The question arises as to whether it is an antibody: its chemical properties suggest that this is rather unlikely. Moreover, it has been shown recently that relatively simple carbohydrates related to those contained in the gonadotrophins also inhibit the effect of HCG but not of HFSH or oestrogens. These carbohydrates are of relatively low molecular weight and are not antibodies.

**Human luteotrophin (prolactin)**

2.12 In the human this hormone appears to be closely related chemically to growth hormone. However, very recently it has been claimed that the two are separable. The development of an immunoassay is awaited with interest but more biochemical work is required before this will become possible. The biological function of this hormone in the human male and female is largely unknown and studies on its secretion and metabolism may help towards a clearer understanding of its possible role in human reproduction. There is immunological evidence for an extra-pituitary source of growth hormone in the placenta: it is not yet clear whether luteotrophic hormone is produced here as well.

**Steroid-protein antigens**

2.13 Steroid hormones, including certain androgens and oestrogens, may be conjugated to a protein such as bovine serum albumin (BSA). The complexes are antigenic and after suitable absorption with BSA the antisera are relatively specific to the BSA-steroid complexes. The possible use of such sera for diagnostic purposes awaits further study.

**Sex-associated proteins**

2.14 A glycoprotein that behaves electrophoretically as a pre-albumin is excreted by mice and has been found by immunological techniques in the serum as well. There is evidence that it is produced in the liver and is cleared by the kidney. The concentration of this protein in the urine and serum of male mice is three to four times as great as in female mice. The levels in female mice are increased by the administration of testosterone and decreased in male mice following castration. A similar protein has been found in rat urine and serum, which in the urine of male rats consists of two subfractions: a pre-albumin and a globulin. Further work on these proteins is required before their significance can be assessed.

**3. SPERM AND SEMINAL FLUID**

3.1 The importance of immunological phenomena involving male genital products has been well recognized since the turn of the century. Widespread experiments on the production of antibodies against spermatozoa, and the oft-reported reduction in fertility of male and female mammals, including man, following immunization with certain male genital antigens attest to the interest in this subject. There is a suggestive analogy between immunological phenomena and the interaction between eggs and sperm involving fertilizin and antifertilizin in certain invertebrate animals.

**Experimentally induced responses to some of the male genital antigens and their effect on male reproductive function**

3.2 Auto- and iso-immunization with whole testicular tissue or with spermatozoa can induce circulating antibodies, cutaneous hypersensitivity, and aspermatogenesis in several species, especially in guinea-pigs. Similar effects have not been found in the rabbit and there is only a single observation reported in man. The testicular lesions involve exfoliation, first of the more mature spermatogenic elements, and then of spermatocytes and spermatogonia. These lesions can be characterized macroscopically by testicular atrophy and microscopically by infiltration of mesenchymal

mononuclear cells of apparently vascular origin. Damage is specific to the testis and limited to the seminiferous epithelium, which can regenerate naturally in many animals. Circulating antibodies have been detected by techniques such as general and passive cutaneous anaphylaxis, complement fixation, passive haemagglutination, precipitation in gel, and sperm immobilization, although the titres found bear no relation to the degree of testicular damage. Skin reactions include immediate, Arthus, and delayed responses.

3.3 Various antigens appear to be involved in the process of auto-sensitization. Recent work has strengthened earlier evidence that one of these antigens in guinea-pigs is a glycoprotein which is soluble in trichloroacetic acid. Furthermore, this antigen is labile to proteases and sodium periodate, and has an electrophoretic mobility similar to  $\beta_2$ -globulin and a molecular weight between that of 7S and 19S $\gamma$ -globulin. It is highly antigenic in all animals tested, inducing circulating antibodies (not precipitins), delayed hypersensitivity and aspermatogenesis. A second, weaker, antigen is insoluble in trichloroacetic acid, has an electrophoretic mobility similar to  $\beta_1$ -globulin, a molecular weight of less than 60 000, and is relatively stable to proteases and sodium periodate. It evokes precipitating antibodies and delayed hypersensitivity, but not aspermatogenesis unless given over a prolonged period. Both of these antigens are found in the acrosome of the spermatozoon and the idiosome of the spermatid by the method of immunofluorescence.

3.4 Additional studies on the chemistry and biological activity of these and other antigens should be encouraged, since such studies not only serve as models of auto-immune syndromes but may lead to a method of controlling fertility.

#### **Spermatozoa-coating antigen**

3.5 A highly effective antigen that has been called the spermatozoa-coating antigen (SCA) is present in the seminal plasma of man, rabbit and other mammals, irrespective of the presence of spermatozoa. The antigen becomes attached to spermatozoa as a surface coating detectable by immunofluorescence and it cannot be removed by any known technique. Other immunological methods that have been used to demonstrate SCA include complement fixation and passive haemagglutination. SCA is produced by the seminal vesicles and is not present on testicular spermatozoa, e.g., from spermatoceles, or epididymal spermatozoa. It is not dialysable, is resistant to heat up to 80°C, and can be concentrated by gel filtration, but further details of its chemistry are unknown.

3.6 Antibodies to SCA react strongly with seminal spermatozoa and, conversely, antibodies to carefully washed seminal spermatozoa react with

homologous seminal plasma. In immunological reactions SCA shows a high degree of species and organ specificity.

3.7 Immunological reactions for SCA are very sensitive. The presence of seminal plasma in the vagina and on the outer, unwashed, genitalia of women may be detected several days after intercourse. The antigen cannot be demonstrated in the cervical canal, however, under conditions when spermatozoa are demonstrated microscopically. SCA is isoantigenic in the rabbit, but immunized animals of both sexes retain their procreative capacity.

3.8 The biological function of SCA is unknown, although it has been suggested that it could be involved in the processes of motility and capacitation. It is not known at present whether SCA is still present on the surface of capacitated spermatozoa. However "non-coated" sperm from several species, including man, are capable of fertilization.

3.9 An unexpected finding recently reported concerns the recognition of a remarkably poisonous substance, which has been named "cobayin", in the seminal plasma of the guinea-pig. The chemical nature and the biological significance of the substance is unknown. Further work on cobayin, on SCA, and on other constituents of seminal plasma and the adnexal glands will be awaited with interest.

#### **Experimentally induced responses to certain male genital antigens and their effect on female reproductive function**

3.10 High titres of antibodies against spermatozoa have been obtained in female experimental animals with or without the use of adjuvants. The effect of these antibodies on fertility appears to be variable: some strains of mice, guinea-pigs and rabbits become less fertile and others sterile. Failure to produce sterility consistently may be due to the low titres of antibody sometimes found in the uterine fluid, even when the serum titre is high. Moreover, the experimental procedures utilized have not been uniform. Epididymal spermatozoa and seminal spermatozoa as well as frozen and thawed extracts of semen have been used for immunization. The amount of antigen injected, the schedule of immunization, and the length of immunization have been varied. There are also several different tests employed to measure the titre of the antibodies produced. The sperm agglutination method measures predominantly the antibodies reacting with surface antigens, whereas the haemagglutination method also measures intracellular antigens. The role, if any, of intracellular antigens in fertilization is still not known.

3.11 In a recent study, immunized female mice with reduced fertility could be classified into two groups. In the first group large numbers of

spermatozoa were found in the uterus but evidently insufficient numbers reached the site of fertilization in the fallopian tubes so that the fertilization rate was low. These mice usually had high levels of circulating antibodies. In the second group spermatozoa were not found in the uterus and the level of circulating antibody was low. In the former group the reduction in fertility was probably due to agglutination, immobilization or lysis of the spermatozoa, perhaps in the fallopian tubes, while in the latter the reduction in fertility was probably due to other phenomena, perhaps uterine anaphylaxis or phagocytosis of spermatozoa.

#### **Naturally-occurring auto-antibodies against male genital antigens**

3.12 It has recently been reported that spermatozoa react with normal sera from adult males and females of every mammalian species so far examined, but in man these reactions are weak. Their immunological nature is suggested by the following concordant observations: (1) immunofluorescence is located on the acrosome, (2) mixed antiglobulin and immune adherence is seen on the sperm head (probably the acrosome), (3) the spermatozoa agglutinate by their heads, (4) there is rapid lysis of the acrosome, and (5) there is complement fixation. Similar reactions also occur between fresh serum and immature germ cells, all of which are lysed within seconds when exposed to autologous or homologous fresh sera. These germ cells give positive immune-adherence in reaction mixtures with fresh serum. The titres against spermatozoa and against immature germ cells are similar in normal serum, and the two reactions might involve similar components of the serum. The serum factor seems to be absent or weak in newly-born rabbits, rises to its maximum titre (approximately  $1/64$ ) at 10 weeks of age in the rabbit, is not altered by immunization, probably does not cross the placenta and can be absorbed from serum by spermatozoa. Further investigation of this phenomenon is required. The observed lysis of immature germ cells might be involved in the induction of auto-immune aspermatogenesis.

#### **Clinical studies**

3.13 The agglutination of motile spermatozoa in the human ejaculate can be due to the action of an auto-antibody against sperm. This sperm-agglutinin is found in seminal plasma as well as in blood serum, where titres are often higher than in seminal plasma. The agglutination physically prevents the spermatozoa from penetrating into the cervical mucus. The presence of auto-antibodies to human spermatozoa has also been detected using the haemagglutination method and employing tanned erythrocytes sensitized with the supernatant of saline extracts of frozen-thawed spermatozoa.

3.14 It has been suggested that, following obstruction of the vas deferens, sperm accumulate in the tubules and subsequently find their way into the interstitia of the vas and epididymis, thus inducing the production of circulating antibodies. Consistent with this hypothesis is the fact that some vasectomized males develop sperm agglutinins in their sera. Although absorption of spermatozoa from the epididymis is probably a normal process, the production of sperm agglutinins does occur but is very rare in non-vasectomized males. Another suggestion is that some inflammatory process, analogous to the supposed action of Freund's adjuvant, may accompany the absorption of the sperm to bring about the production of significant amounts of antibody. It has not yet been proved that aspermatogenesis or hypospermatogenesis due to auto-immunization can occur naturally in man. At present there is no known form of treatment for patients with auto-agglutination of their sperm.

3.15 Iso-antibodies to spermatozoa have also been reported to occur in the sera of some infertile women. Such antibodies have not been detected by passive haemagglutination tests in the sera of fertile women or virgins. However, spermagglutinins occur in the sera of many infertile and a few fertile women. Reports on their significance in relation to infertility are conflicting. It may be that the rates of passage of antibodies from the serum to the genital tract vary considerably.

3.16 Anaphylactic reactions to semen have been reported occasionally in women following intercourse. Reagins to seminal plasma have been detected in the sera of such subjects.

#### **Antagglutinins**

3.17 The so-called antagglutinins are said to occur in the seminal plasma of several mammalian species. They have been shown to inhibit the spontaneous auto-agglutination of washed spermatozoa in salt solutions. The biological significance of these antagglutinins is not clearly established: they may perform an important function in the seminal plasma and also in the female genital tract.

### **4. BLOOD GROUP ANTIGENS AND HUMAN REPRODUCTION**

4.1 Recent progress in the study of blood groups in man has made it possible to identify some 80 different antigens on the red blood cells, all of which are determined by dominant effects of the related genes. By appropriate analyses they are divided into more than a dozen systems such as ABO, Rh, MN, Xg, etc. Some of these antigens have been found

in other cells of the body and have been repeatedly involved in problems of fertility and foetal pathology.

#### **Blood group antigens and the germ cell**

4.2 The phenotypic expression of haploid characters in spermatozoa would lead to the production of varied types of spermatozoa from a single male, e.g., an AB heterozygote would produce both type A and type B spermatozoa. Immunological techniques could then be used to identify and to separate specific types of spermatozoa. The antigens, if present, could lead to gametic selection in the uterus against incompatible types of spermatozoa. Obviously gametic selection would involve only those surface antigens determined by the haploid genotype of the spermatozoon, and not those absorbed to the surface of the spermatozoon from secretions in the male or female genital tracts.

4.3 The presence of blood group antigens on spermatozoa was reported before it was realized that the secretor status of the donor, as determined by the presence or absence of A and B antigens in the saliva, should be taken into account. More recent studies show that in man, A and B antigens are present on spermatozoa from secretors, but not from non-secretors. In AB secretors, however, it has not been possible to distinguish two types of spermatozoa. These antigens are evidently absorbed from the genital environment and they are not removed by extensive washing. In this respect they resemble SCA (see sections 3.5-3.9) but unlike the latter are secreted throughout the male reproductive tract since they have been detected on spermatozoa from spermatocoele.

4.4 Neither Rh nor the sex-linked antigen Xg<sup>a</sup> have been detected on human spermatozoa while tests for M, N and Tj<sup>a</sup> give weakly positive results, although some unexplained reactions between M and N have been recorded. Human spermatozoa have also been shown to possess an antigen common to the HeLa cell.

4.5 In support of these findings is the observation that in patients with high circulating titres of antibodies to spermatozoa no reaction against the donor's specific blood group antigens could be recorded.

4.6 Neither A nor B antigens have been detected on rabbit spermatozoa, while in mice, neither H2<sup>d</sup>, H2<sup>k</sup> nor other histocompatibility antigens could be detected on spermatozoa by mixed antiglobulin reactions or by second set rejection of skin grafts; previous studies in rabbits by the latter method gave the same results. Antibodies made in one inbred line of mice against spermatozoa from a second inbred line failed to discriminate between the two types of spermatozoa. Thus, the existence of strain-specific antigens on spermatozoa has not been proved at present.

**The effect of blood group incompatibility on human fertility**

4.7 Since most blood groups are widely distributed polymorphic traits and have high gene frequencies in some populations, segregation analyses of family data in such populations should reveal the various parameters involved in the effect of blood group incompatibility on human reproductive performance. These analyses can be done using data obtained by precise laboratory procedures and by using refined statistical methods for discriminating between gametic and zygotic selection.

4.8 Although the number of offspring incompatible with the mother resulting from ABO incompatibility between the parents has frequently been reported to be lower than expected, recent data in one country have shown that such incompatibility had no detectable influence as judged not only by the segregation ratio, but also by the mean number of pregnancies, the proportion of foetal deaths per pregnancy, the proportion of childless couples, and the mean number of living children per marriage. Only when considering the percentage of sterile couples was a slight but significant increase observed among incompatible matings. These conclusions differ from those obtained from a similar analysis made in the same country using data collected before 1945 when the frequency of children incompatible with their mothers was found to be lower than expected. Similar discrepancies with comparable sets of data from other countries have been noted. No clear explanation of these discrepancies has yet been found.

4.9 The secretor status for ABH antigens is another factor that could influence reproduction. This status can be classified easily and shows simple Mendelian segregation. In a recent extensive study of nearly 12 000 persons no differences could be found however in the percentage of sterile couples, the mean number of pregnancies, the incidence of abortions, and the interval between the initiation of cohabitation and the first live birth in compatible or incompatible matings for secretors. Furthermore, no gametic selection for secretor genes could be demonstrated by means of refined analysis of segregation ratios. The reliability of the testing procedure is indicated by the fact that only four "aberrant secretors" were detected among the persons tested. The result of this survey suggests that the secretor status is not playing an important part in reproductive performance. Further studies are necessary. Detailed investigations should be extended to other populations and areas to see if the results observed so far have general validity.

4.10 The well-established harmful consequences of incompatibility between mother and foetus with respect to the Rhesus blood group antigens are recognized as among the clearest examples of the immunological effects of blood group incompatibility on human reproductive performance.

Certain aspects of the mechanisms that may be involved in protecting the foetus against maternal Rh antibodies as well as other blood group antibodies are referred to in sections 5.18-5.21. The Group felt, however, that the problems of treatment and prevention of Rh haemolytic disease merit more detailed discussion at a later date.

## 5. MATERNAL-FOETAL IMMUNOLOGICAL INTERACTIONS

5.1 Explanations of the immunological interactions between mother and foetus are still largely hypothetical. A better understanding of the mechanisms involved would be desirable and of great practical interest.

### Placentotrophin

5.2 During pregnancy, oestrogens are produced in relatively vast quantities and, as is well known, they inhibit the release of pituitary gonadotrophins. It has been reported that, during pregnancy, oestrogens also stimulate the secretion of another pituitary hormone, placentotrophin. This is stated to be produced also by non-pregnant women given prolonged and intensive treatment with oestrogen. Placentotrophin has been extracted from the urine of pregnant women and appears to be a glycoprotein having a molecular weight of about 36 000. Its function is to stimulate the placenta to produce HCG and it has been found to have the same effect on transplanted trophoblast in the absence of oestrogen.

5.3 Mechanisms may exist during pregnancy whereby the formation of antibodies to the foetal antigens is normally suppressed. These could depend upon the increased production of corticosteroids or upon other factors including those recently suggested to account for experiments involving homotransplantation of trophoblast and foetal skin into non-pregnant individuals. These experiments clearly demonstrate that the embryonic tissues are antigenic and their grafts disappear within a few weeks. However, following the administration of oestrogens, or of placentotrophin, the transplanted trophoblast is stimulated to produce HCG, and there is intense proliferation of the epithelium and no immunological tissue reaction. Further observations on the nature and activity of placentotrophin will be awaited with interest since confirmation of these preliminary claims could provide a new means of controlling immunological reactions during homotransplantation.

### Host-against-graft reactions

5.4 In spite of numerous investigations, a host-against-graft rejection reaction of a mother to her foetus *in utero* has not been clearly demonstrated.

The grafting of incompatible paternal skin to mice does not affect the course of pregnancies, despite the rejection of the skin graft. This shows also that the graft rejection is not prevented by pregnancy.

5.5 In a recent study, intact mouse and rabbit blastocysts were scored for the presence of transplantation antigens, using mixed agglutination or mixed antiglobulin tests. The zona pellucida of the blastocysts was first removed with pronase, a proteolytic enzyme that does not destroy these antigens in spleen cells. In both species, group B antigen could be detected on the blastocysts. In mice, neither H2<sup>a</sup> nor H2<sup>k</sup> were detected on the trophoblast, which could explain why no host-versus-graft rejection reaction occurs at implantation.

5.6 Some interesting attempts to devise a more delicate test than actual rejection have been reported. In the first of these the inbred and cross-bred mouse foetuses in inbred mothers were compared for spleen and placental size, the supposition being that a rejection reaction might be detected by enlargement of the spleens and reduction of the placental size of the cross-bred foetuses as compared to the same organs of the inbred foetuses. In fact the opposite was found to occur. Moreover, this effect was not due to heterozygosity, since increase in placental size occurred with inbred foetuses after transplantation into the uteri of mothers of another strain. Secondly it was found that in C57BL mothers immunized to A<sub>2</sub>G spleen cells the placentae of A<sub>2</sub>G foetuses were larger than in untreated mothers. Thirdly, ectoplacental cones of mouse embryos were transplanted into the testes of adult mice. Trophoblast grafted in this way was more actively invasive when it was derived from an inbred strain different from that of the host than when it was from the same inbred strain.

5.7 Since the size that the placenta can attain depends initially on the extent of the trophoblastic invasion of the uterine mucosa, it seems probable that the increase in size of the placenta when the foetus differs antigenically from the mother is due to a more extensive initial invasion of the trophoblast. It is suggested that the initial reaction of the host tissue to the antigenically dissimilar trophoblast is in the nature of an inflammatory response, accompanied by oedematous and hyperaemic swelling of the tissues, thus permitting a deeper penetration of the invading trophoblast. Further investigation of the histological and cytological changes occurring in the placenta is required.

#### **“ Stage-specific ” antigens**

5.8 In chick embryos, specific antigens for each organ (the so-called organ-specific antigens) appear successively during development but in

the rat they appear over a brief period of time, as judged by the development of lens antigen. There is some evidence that human antigens resemble those of the rat in this respect. Another class of embryonic antigen of ephemeral duration has been described and is termed "stage-specific". The appearance of stage-specific antigens coincides approximately with the appearance of the anlagen of the particular organ: they are found also in regeneration processes of specific organs in adults.

5.9 The stage-specific antigens have been demonstrated and differentiated from antigens specific for normal adult organs by anaphylactic or hypersensitivity reactions in the guinea-pig. Precipitin methods in agar gel and complement fixation have been used to demonstrate what appear to be stage-specific antibodies in the sera of women at 14-15 weeks of pregnancy, which is shortly after the appearance of stage-specific antigens in the foetus. Although these antibodies have been reported in all normal pregnancies studied, no anti-heart antibodies were found in the sera of certain pregnant women giving birth to babies with functional heart disorders: other antibodies to organs such as kidney, which were normal in these offspring, were present in the maternal sera.

5.10 In further experimental studies, serum was removed from normal, non-pregnant rats and from rats on the 10th day of pregnancy, and in two similar groups of rats one lung was extirpated 48 hours before removal of the serum. These sera were then passively transferred into normal pregnant rats on the 8th to 9th days when the rudiments of the foetal lungs were beginning to develop. It was noted that a specific increase in the size of the foetal lung occurred only in rats of the group inoculated with serum from the pregnant rats from whom one lung had been extirpated, although sera from normal non-pregnant rats gave a generalized increase in the size of foetal organs. Sera from normal pregnant rats did not increase the size of foetal organs, nor did sera from non-pregnant rats that had had one lung extirpated.

5.11 Because of the important implications of these experiments, their confirmation in other laboratories is highly desirable. Further experiments are called for using lines and fractionated serum to determine the relationship of this stimulating factor to stage-specific antibodies.

#### **Transmission of immunity from mother to foetus**

5.12 Although extensive information is available on the transmission of immunity from mother to foetus in experimental animals there is much still to be learned about the process in man. It is known that in the rabbit and other animals, immunoglobulins are absorbed by the endoderm of

the yolk-sac from the uterine lumen and transmitted to the foetal circulation, while in the rat and mouse, transmission is mainly after birth by way of the gut. Clearly the route of transmission is not by way of the yolk-sac in women since this organ is vestigial, and although anti-Rh antibody has sometimes been found in the amniotic fluid of babies suffering from haemolytic disease, transmission is generally considered to be by way of the placenta. Since this organ is so different in embryonic origin and structure from the foetal yolk-sac or neo-natal gut, it would be unwise to assume a close similarity of function.

5.13 As in the rabbit, transmission of immunity in women occurs only during pregnancy. The transmission is selective, as it is also in other species, the clearest example of this being the transmission of blood-group antibodies, complete agglutinins being transmitted at very low levels or not at all, whereas incomplete agglutinins are transmitted readily. This difference in transmission may be related to differences in the distribution of the immunoglobulin fractions, 7S globulin being transmitted while 19S macroglobulin is not.

5.14 During transmission, absorption by the cells is probably pinocytotic and non-selective in the rat and rabbit. Selection appears to take place after absorption and before secretion into the circulation, any excess protein being degraded.

5.15 Delicate methods are now available for determining the precise concentration of purified immunoglobulin fractions in the cord blood as compared to the maternal blood. This has been made possible by the important advances in the separation and purification of the serum proteins and the use of isotopic labelling, and these techniques should be exploited.

5.16 The immunoglobulin molecule can be split into fragments carrying the antigen and antibody activities by digestion with enzymes such as papain or pepsin. Originally three fragments were recognized: two carried the antibody activity, while the third was antigenic and had no antibody activity. The antibody components are referred to as the Fab-fragments and the antigenic components as the Fc-fragments. In animal experiments with isotopically labelled fragments it is found that the Fc-fragments are transmitted more readily than the Fab-fragments. It would be interesting to know if the same holds for man: this would have an important bearing on the passive immunization of the mother during pregnancy and its possible effect on the foetus. Moreover, it is found that immunoglobulins of one species, or their Fc-fragments, interfere with the transmission of antibodies in another species: it is not yet known if a similar mechanism exists in man.

5.17 There is an interesting resemblance between the placental transmission of immunity and anaphylactic sensitization. Both processes are selective and specific receptors of immunoglobulin are probably involved, the molecule being attached by the Fc-piece. Further investigation along these lines in a single species, such as the guinea-pig, should lead to a better understanding of the functioning of immunoglobulin.

#### **Protection against maternal isoantibodies during human pregnancy**

5.18 The fact that normal gestation is the rule in spite of the immense frequency of isoantigenic blood group incompatibility between mother and foetus seems to imply the presence of some kind of protective mechanism. In ABO incompatibility it has been reported that the titre of agglutinins against the foetal antigen, absent in the mother, is considerably reduced in the maternal placental blood as compared with the maternal peripheral blood; this phenomenon has not been found for agglutinins against antigens absent in the foetus. It has also been reported that the decidua and the chorionic membranes contain isoantigens of maternal origin only, while amniotic membrane and amniotic fluid contain isoantigens of foetal origin only.

5.19 On the basis of these findings an interesting working hypothesis has been proposed according to which isoagglutinins that might be otherwise detrimental to the foetus would be adsorbed on isoantigens from the amnion and amniotic fluid. In order to test this hypothesis, an experimental model for haemolytic anaemia has been developed in the monkey. Males possessing what appears to be a special blood group antigen were mated with females naturally lacking this antigen but immunized with it during pregnancy. The antigen has been characterized as a soluble mucopolysaccharide and has been named "Ako". The offspring displayed symptoms resembling those of haemolytic disease in newborn children.

5.20 The soluble form of the antigen was next injected into the amniotic fluid of several foetuses with the object of adsorbing maternal antibodies. Preliminary results are encouraging and further experiments are in progress.

5.21 Because of the important implications of this experimental model for an understanding of the etiology and treatment of haemolytic disease of the newborn in man, the Group believed that more extensive studies along similar lines should be undertaken in several laboratories.

## 6. RESEARCH NEEDS

6.1 The Scientific Group considers that further investigations are required of the problems listed below. They are not arranged in any particular order of priority. The references in parentheses indicate the relevant passages in the body of the report.

1. The correlation between physicochemical, biological and immunological criteria for the purity of antigens concerned in human reproduction (1.2; 2.2; 2.5; 2.7).

2. The chemical structure of hormones concerned with reproduction, with special reference to the biologically active sites and the nature of antibodies against these active sites (1.3; 2.1; 2.3; 2.5; 2.8; 2.11).

3. Production of antibodies to the gonadotrophins by the use of adjuvants and/or chemically modified gonadotrophins (2.10).

4. Modification of hormones from other species to render them active but non-antigenic in man (2.10).

5. The use of immunological methods for assisting in the detection of the time of ovulation: these could aid in the control of fertility and in the treatment of infertility (2.6).

6. The development of strains of animals of high immunological competence.

7. Characterization of the male antigens responsible for various immunological phenomena in males, particularly the agglutination of spermatozoa by circulating or seminal antibodies, delayed hypersensitivity and the production of auto-immune aspermatogenesis (3.2 et seq.).

8. Characterization of male antigens responsible for inducing circulating antibodies and reducing the fertility of immunized females (3.10 et seq.).

9. The further characterization of natural auto-antibodies said to be responsible for reactions observed between normal serum and certain male antigens, and the clarification of their relations to other immunological phenomena (3.12).

10. The risks involved in immunization with antigens concerned with reproduction (3.2).

11. Comparison of various methods of detecting spermatocidal antibodies in the sera of infertile males and females (3.13).

12. The treatment of patients with sperm auto-agglutinins (3.14).

13. The search for auto-immunity to testicular antigens in patients with spermatogenic disorders (3.13 et seq.).

14. The nature and biological significance of the antagglutinins (3.17).
  15. The location of blood group antigens on spermatozoa and on blastocysts (4.2 et seq.).
  16. Mechanisms and types of antibodies involved in foetal damage (4.9 ; 4.10).
  17. Possible ways of interfering with the transmission of antibodies in man, e.g., in haemolytic disease (5.16).
  18. The relationship of differential fertility to blood groups in different populations and areas and its effect on succeeding generations (4.7 et seq.).
  19. The chemistry and biological activity of placentotrophin (5.2 ; 5.3).
  20. Stage-specific antigens and their relationship to foetal development (5.8 et seq.).
  21. The mechanism of the transmission of immunity from mother to foetus in man (5.12 et seq.).
  22. The possible occurrence of specific anti-trophoblastic antibodies pre- and post-partum (5.4 et seq.).
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