THE TRANSFER OF ANTIBODY FROM MOTHER TO FOETUS IN THE GUINEA-PIG

Thesis submitted for the Degree of
Doctor of Philosophy
of The University of Leeds
by

Maha Raouf Al-Najdi
B.Sc. (Baghdad), M.Sc. (Tallahassee, Florida).

Department of Bacteriology
University of Leeds

October 1965

CONTAINS

PULLOUTS

CONTENTS

	Page
ACKNOWLEDGMENTS	
INTRODUCTION	1
LITERATURE REVIEW	4
Postnatal (colostral) transfer of immunity..	4
Pre- and postnatal transfer of immunity	46
Prenatal transfer of immunity	61
Miscellaneous	98
EXPERIMENTAL METHODS	99
RESULTS	140
DISCUSSION	171
SUMMARY	193
REFERENCES	196

ACKNOWLEDGMENTS

I wish to express my thanks and gratitude to Professor C.L. Oakley, F.R.S., for suggesting the problem investigated, and for his interest, generous help and advice throughout the course of this study and the preparation of this manuscript. I would like to thank him for his supply of heterologous antitoxins essential for this study and for his pepsin-digestion of the guinea-pig tetanus antitoxin.

I would like to thank Professor F.W. Rogers Brambell, F.R.S., for allowing me to visit his laboratory and Dr W.A. Hemmings for demonstrating the technique of intra-uterine injections and collection of samples in the rabbit.

I would like to thank all members of the Department of Bacteriology and especially Mrs Y. Robinson, for their help in many aspects of my work.

I wish to thank Miss E.M. Reed and her staff at the Leeds Medical School Library for their co-operation and Mrs M. Kidd for typing and reproducing the thesis.

I wish to thank my colleagues Misses S. Ancrum and E. Chamberlain for their help in the translation of the French literature and for their criticism.

This work was carried out while I was holding a scholarship from the Iraqi Government.

INTRODUCTION

In their early development and before their antibody-forming mechanism is fully established the young of mammals depend entirely on the maternal supply of antibodies. The passive transfer of immunity acquired by the young from its mother can occur before birth, after birth, or both. In man, rabbit and guinea-pig, for example, the transfer of immunity from mother to young occurs entirely prenatally. In the ungulate, horse and pig the passive transfer of immunity occurs postnatally, whilst in dog, rat and mouse it occurs both pre- and postnatally.

The difference between these groups in the mechanism of antibody transfer from mother to young was earlier thought to be due to the difference in the number of layers intervening between the maternal and the foetal circulations (Kuttner and Ratner, 1923). In other words the structure of the placenta was the decisive factor in the mechanism of the transfer of passive immunity to the young.

According to Grosser's (1909; 1927) classification, there are four types of mammalian placental structure. (1) the epitheliochorial with six intervening layers, (2) the syndesmochorial with five, (3) the endotheliochorial with four and (4) the haemochorial placenta with three. These were later modified by

Mossman (1926), who added a fifth type, the haemoendothelial with one.

Since animals with epitheliochorial and syndesmochorial placentae transfer antibody entirely postnatally to their young, whereas in those with haemochorial and haemoendothelial placentae transfer is mainly prenatal (with the exception of rats and mice where transfer is mainly postnatal), and in those with endotheliochorial placentae both prenatal and postnatal transfer is considerable, it was perhaps not surprising that a purely physical hypothesis of antibody transfer should be generally accepted.

But in 1948 Hartley showed that the human placenta was selective in transferring antibody, so that refined horse antibody was not transferred to the foetus at all, while human antibody passed freely. Shortly after, in a series of papers, Brambell and his colleagues (1949, 1950, 1951, 1952) showed that there was considerable doubt whether in rabbits antibody is transferred via the placenta at all. They suggested that antibody circulating in pregnant rabbits is secreted into the uterine cavity, whence it passes via the yolk-sac splanchnopleur into the foetal circulation. Moreover they showed that the yolk-sac splanchnopleur is a highly selective membrane, differentiating between -globulins

not by molecular size but by species of origin. In particular Brambell, Hemmings and Oakley (1959) showed that rabbit antibody digested with pepsin was less readily transferred to the foetus, notwithstanding its lower molecular weight, than the unmodified antibody. When rabbit -globulin was digested with papain (Brambell, Hemmings, Oakley and Porter, 1960) to yield Porter's fractions I, II and III and these fractions were injected into the rabbit uterine cavity, fraction III was transferred to the foetus almost as well as the unmodified -globulin, fraction I and II much less well.

In the guinea-pig the route of antibody transfer from mother to young was also shown to be via the yolk-sac splanchnopleur and the vitelline circulation of the foetus (Barnes, 1957).

The present work was undertaken to investigate by the technique of intra-uterine injections of antitoxic sera used by Brambell and colleagues the selectivity of the yolk-sac splanchnopleur of the guinea-pig foetus for homologous and various heterologous antitoxins and for the antibody fragments obtained by the peptic digestion of the antitoxin molecule.

LITERATURE REVIEW

POSTNATAL (COLOSTRAL) TRANSFER OF IMMUNITY

Transfer of immunity from mother to young in the horse, pig, sheep, cow and goat occurs almost entirely in the first few hours of postnatal life via the ingestion of colostrum. Famulener (1912) was apparently the first to study the role played by colostrum in the transfer of immunity; he showed that kids acquired little if any immunity *in utero*; passive immunization of newborn kids was chiefly colostral. The following review is concerned with the passive transfer of immunity in the horse, pig, sheep, cow and goat and the role played by colostrum.

Before discussing the transfer of passive immunity to the young it was thought necessary to consider in brief the early development and the arrangement of the foetal membranes and placentation. So far as possible the foetal membranes in contact with the uterine wall or exposed to its lumen were taken into account.

THE HORSE

Foetal membranes and placentation (largely based on Mossman, 1937)

The blastocyst, with a bilaminar omphalopleur, remains unattached to the uterine wall until relatively late in development at about the tenth week (Fig. 1).

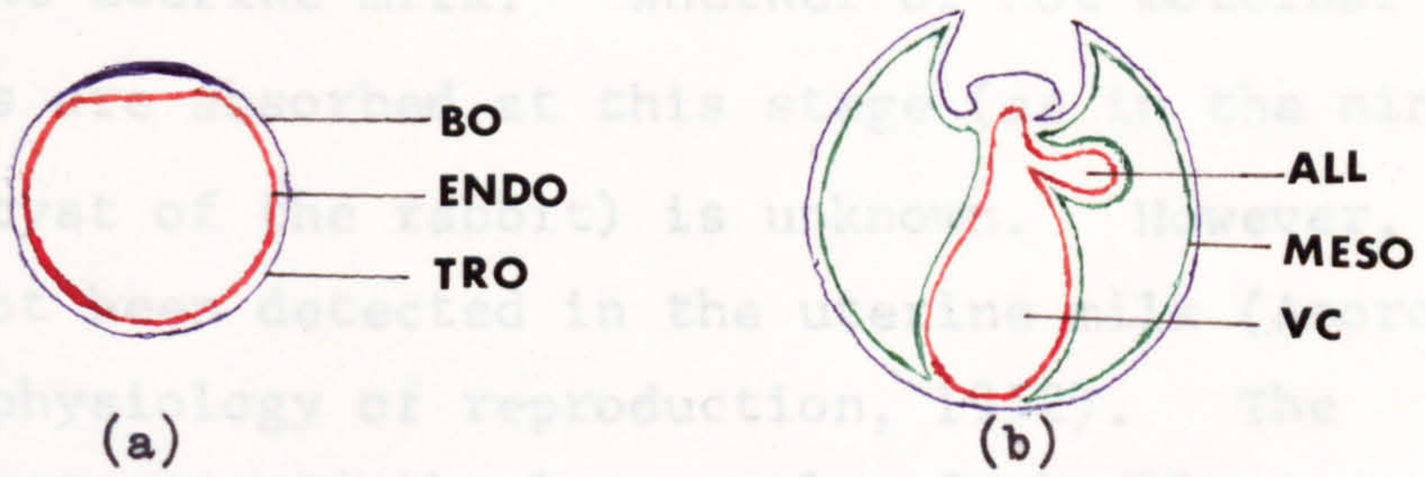


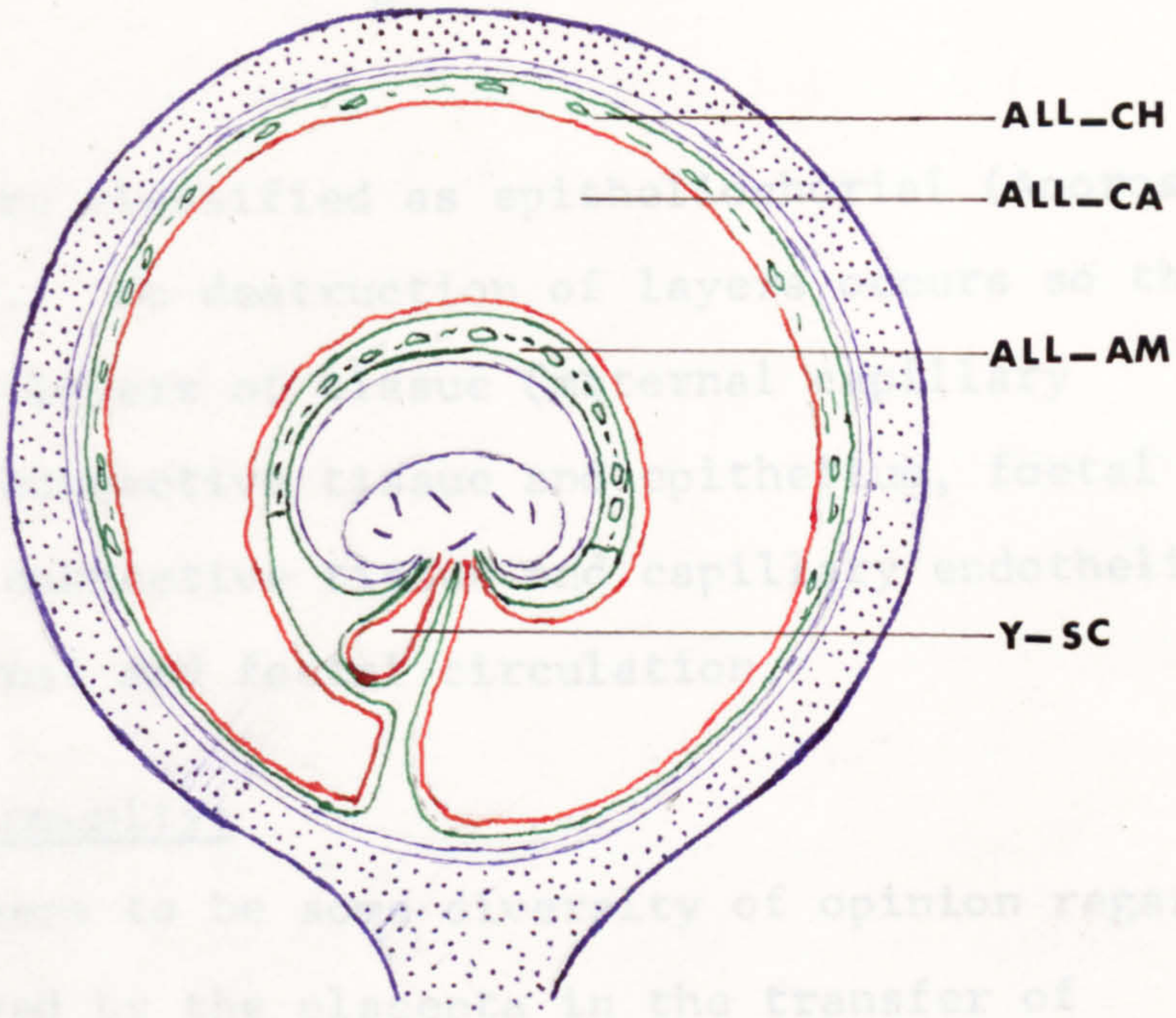
Figure 1

Blastocyst and early development of embryonic membranes of horse

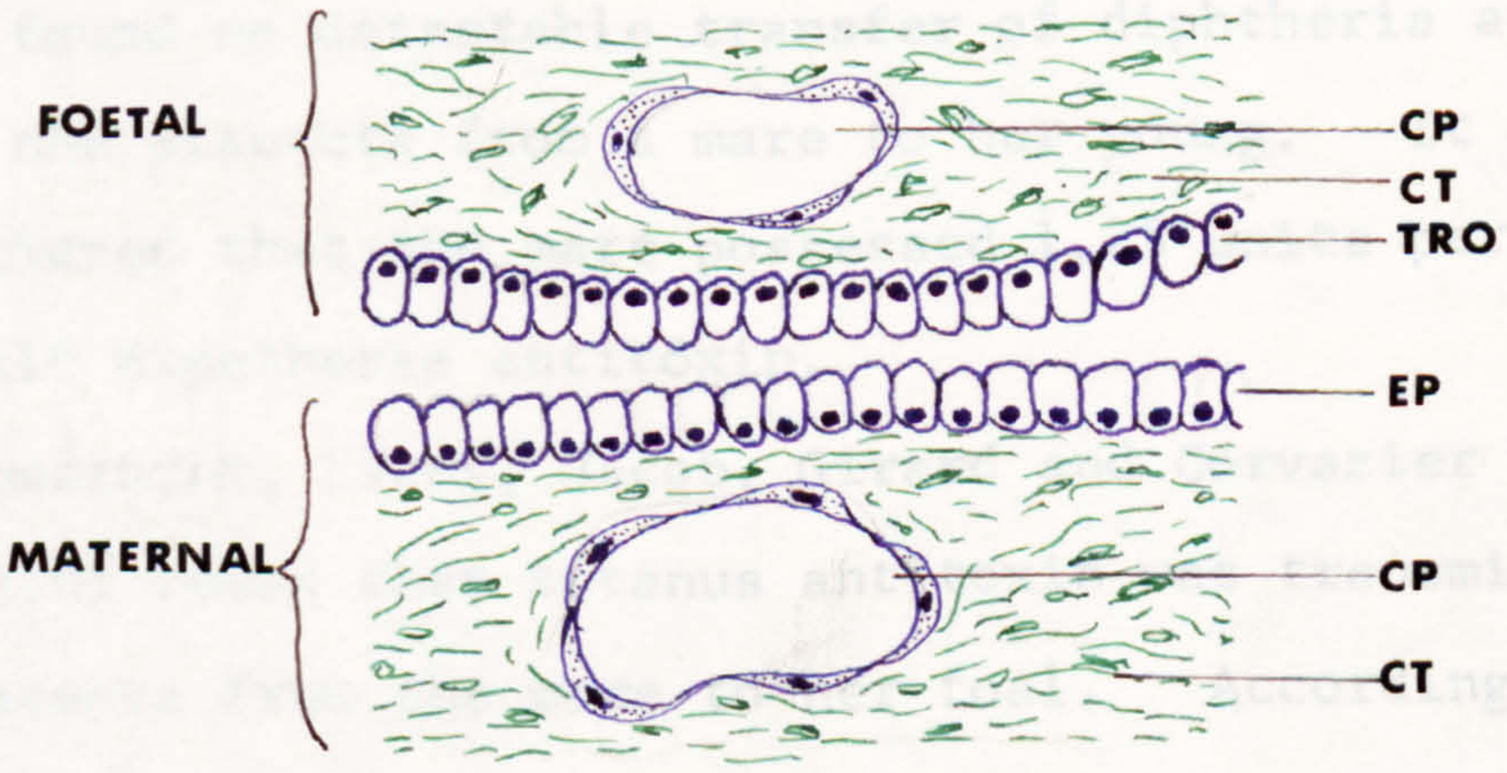
ALL, allantois; ALL-AM, allanto-amnion; ALL-CA, allantoic-cavity; ALL-CH, allanto-chorion; BO, bilaminar omphalopleur; ENDO, endoderm; EXO, exocoelom; MESO, mesoderm; TRO, trophoblast; UM, uterine-muscle; VC, vitelline cavity. (a and b are hypothetical; c is after Missman, 1937).

While free in the uterine lumen the blastocyst is nourished by the uterine milk. Whether or not maternal plasma proteins are absorbed at this stage (as in the nine-day-old blastocyst of the rabbit) is unknown. However, globulin has not been detected in the uterine milk (Amoroso in Marshall's physiology of reproduction, 1952). The mesoderm does not grow to the lower pole of the blastocyst until late in pregnancy, and as a consequence the bilaminar omphalopleur and a chorio-vitelliue placenta are both present for some time. The exocoel is formed by cavitation in the mesoderm. The amnion is formed by folding of the germ layers. The allantoic vesicle then develops mainly dorsally in the direction of the abembryonic hemisphere and practically enclosed the yolk-sac; in its later stages it compresses the exocoel and vitelline cavity so that at term neither is detectable (Fig. 2). The inner wall of the allantoic vesicle fuses with the amniotic mesoderm and the peripheral amnion to form a vascularized allanto-amnion; its outer wall fuses with the chorion to form the chorio-allantoic placenta. The membranes exposed to the uterine cavity are therefore in turn: bilaminar omphalopleur, chorion and bilaminar omphalopleur, allantochorion.

Implantation is of the central type; the placenta is diffuse with short branched villa formed as a simple apposition of chorionic and endometrial epithelial surfaces;



(a)



(b)

Epitheliochorial placenta

Figure 2

Late development of embryonic membranes and placenta of horse

CP, capillary; CT, connective tissue; EP, epithelium; Y-SC, yolk-sac; for details of ALL-AM, ALL-CA, ALL-CH and TRO, see figure 1. (a is after Mossman, 1937; b is after Amoroso, 1952).

it is therefore classified as epitheliochorial (Amoroso, 1961)(Fig. 2). No destruction of layers occurs so that there are six layers of tissue (maternal capillary endothelium, connective tissue and epithelium, foetal trophoblast, connective tissue and capillary endothelium) between maternal and foetal circulation.

Transfer of immunity:

There seems to be some diversity of opinion regarding the part played by the placenta in the transfer of antibodies from mare to foal. Mason, Dalling and Gordon (1930) found no detectable transfer of diphtheria antitoxin across the placenta from a mare to her young. It should be mentioned that the mare possessed 1.25 units per ml. of "natural" diphtheria antitoxin.

Lemétayer, Nicol, Jacob, Girard and Corvazier (1946 a,b) found that tetanus antitoxin was transmitted via the placenta from the mare to her foal. According to them, the immunity in the mare must attain a certain level (one unit per ml. for tetanus) for the placental transfer to be detected. However, they said that the colostral immunity played a much more important role in the passive immunity of the foal.

Caroli and Bessis (1947 a) reported the presence of

the haemolytic disease in newborn mules due to the immunization of the mare against the blood group substances of the foetal mule (inherited from the donkey) and the transfer of these antibodies to the newborn mule. According to them, the mule was born healthy and the disease occurred only after several feeds from the udder of the immune mare. In another article Caroli and Bessis (1947b) implicate the placenta as the route of transfer of the mare anti-mule red blood cells to the foetal mule.

Millot and Gorius (1950) also studied the haemolytic disease of newborn mules; they found that the colostrum antibody titre was higher than that of the serum. These authors implicated the colostrum as the main factor of the haemolytic disease of newborn mules. As prophylactic measure they suggested preventing the mule from sucking the colostrum.

Studies on the serum fractions of foal have been made by few authors. Earle (1935) found that euglobulin was either absent or present only in very small quantities in newborn foal's serum. Pseudoglobulin 1 was also present in small quantities. After the ingestion of colostrum there was an increase in euglobulin and a marked increase in pseudoglobulin 1.

Polson (1943) found by electrophoretic examination that the serum of the newborn foal before colostral feeding is characterized by high albumin and α-globulin concentrations

and by traces of β-globulin; γ-globulin was completely absent from the serum. At five days of age there was a decrease in albumin, a slight increase in α-globulin, and a tremendous increase in β-globulin of the foal serum. However, only traces of γ-globulin appeared in the foal's serum at five days of age.

Pedersen (1945) found that fetuin was the predominant component in the sedimentation diagram of the "total globulin" fraction of the foal's serum.

Absorption of homologous and heterologous antitoxic sera by the intestine of the newborn foal was reported by Mason et al. (1930).

SUMMARY

1. Colostrum plays the major role in the transmission of passive immunity from mother to young in the horse.
2. The foal is born with no γ-globulin in its blood.
3. Fetuin is the major component of the globulins in the newborn foal.

THE PIG

Foetal membranes and placentation:

In the pig the blastocyst is formed of two concentric epithelial layers forming a bilaminar emphalopleur surrounding a vitelline cavity; the blastocyst is free in the uterine cavity (Fig. 3a). At the end of the seventh day of gestation the blastocyst begins to elongate with the accumulation of fluid between the ectoderm and endoderm (Fig. 3b). An embryonic disc develops as a thickening of the blastocyst. Mesoderm from the primitive streak passes laterally between ectoderm and endoderm, and is distended with fluid to fill the gap between them; it forms the chorion by approximation to ectoderm. The yolk-sac is large and very cascular in young embryos; it is for some time fused ventrally with the chorion. The amnion is formed by folding and fusion of layers over the embryo (Fig. 3c); it gradually fuses with the chorion and practically obliterates the central part of the exocoel. The allantois grows out from the gut and gradually pushes the exocoel aside until it comes in contact with the chorion except at its extreme ends to form the allanto-chorion (Fig. 4a); it is well supplied with blood vessels which later invade the amnion chorion area.

The surfaces exposed to the uterine cavity are, like those in the horse, in turn, the bilaminar omphalopleur,

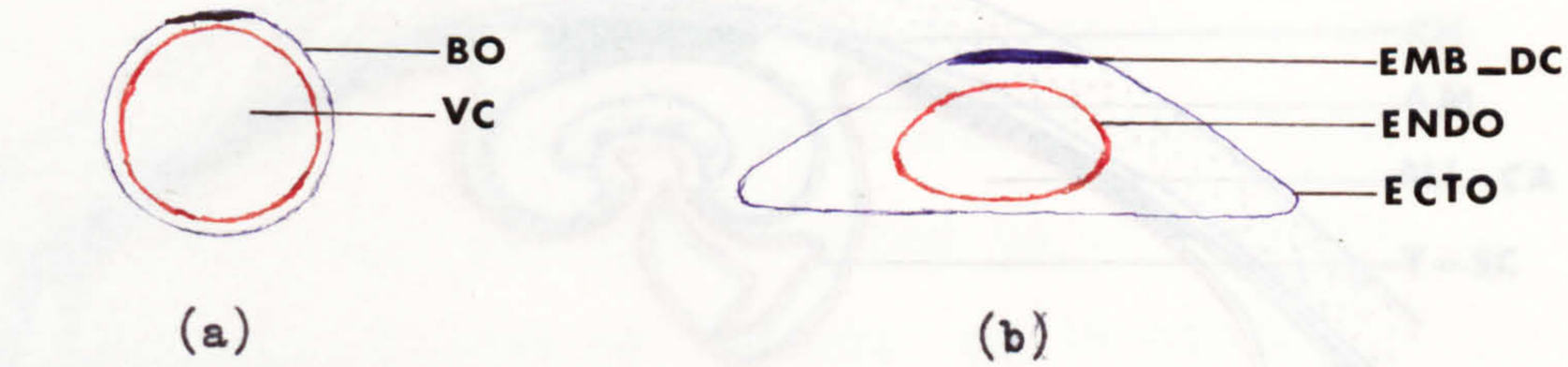
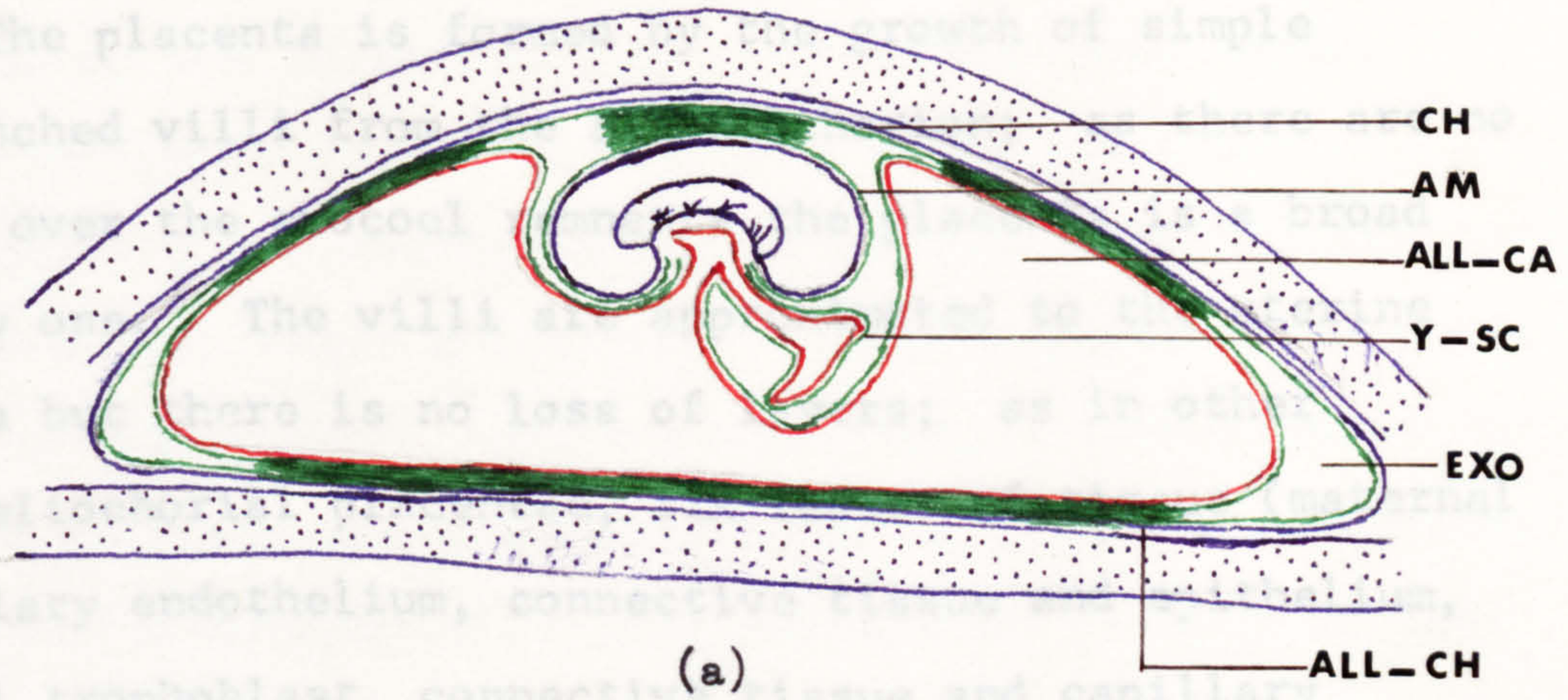


Figure 3

Blastocyst and early development of embryonic membranes of pig

AM-FD, amniotic fold; CH, chorion; ECTO, ectoderm; EMB-DC, embryonic disc; for details of ALL, BO, ENDO, EXO, MESO, and VC, see figure 1. (a, b and c are after Mossman, 1937).



Epitheliochorial placenta

Figure 4

Late development of embryonic membranes and placenta of pig

AM, amnion; CH, chorion; CP, capillary; CT, connective tissue; EP, epithelium; Y-SC, yolk-sac; for ALL-CA, ALL-CH, EXO and TRO, see figure 1. (a is after Mossman, 1937; b is after Amoroso, 1952).

the chorion and bilaminar omphalopleur, the allantochorion.

The placenta is formed by the growth of simple unbranched villi from the allantochorion; as there are no villi over the exocoel remnants the placenta is a broad zonary one. The villi are approximated to the uterine mucosa but there is no loss of layers; as in other epitheliochorial placentae, six layers of tissue (maternal capillary endothelium, connective tissue and epithelium, foetal trophoblast, connective tissue and capillary endothelium) intervene between maternal and foetal circulations (Fig. 4,b).

Transfer of immunity.

There seems to be no transfer of passive immunity from sows to their offspring in utero.

McArthur (1919) found that piglets retained their immunity against hog cholera so long as they were suckled and in most cases for a few weeks after weaning. At that time the route of transfer of immunity from sows to piglets was still a question, but the author suggested the milk as the means of transfer of immunity.

Nelson (1932; 1934) found that immunity against vaccinia virus was transmitted to piglets by the way of colostrum and not by the placenta. This passive immunity acquired by the suckling piglets began to decline during the second month and was negligible by the end of the

third month.

Young and Underdahl (1950) immunized sows against swine influenza by exposure to the virus and found neutralizing and haemagglutination-inhibiting (HI) influenza antibodies in their colostrum. They suggested that these "antibodies" were in part non-specific, but considered that a rise in titre was significant and indicated the presence of specific antibodies. They found that neither of the two antibodies was present in the newborn pig before suckling. High titres of both antibodies were found within thirty hours after suckling and decreased gradually over an eight week period.

The blood serum proteins of piglets before and after colostral ingestion received the attention of many authors using fractionation and electrophoretic examinations in addition to immunological methods. Earle (1935) found that very small quantities of globulins were present in piglets' blood at birth. In one piglet the total globulin rose within twenty four hours to levels six times greater than that in the serum of newborn pigs.

Foster, Friedell, Carton and Dieckmann (1951) made electrophoretic studies on the composition of piglet plasma during lactation. They found that there was an increase in the concentration of γ-globulin in the pig's plasma from a level as low as 1.3 mg. per ml. before suckling to

about 20 mg. per ml. during the first twenty four hours post partum. In these experiments however, the authors worked with pooled blood samples from each litter.

Barrick, Matrone and Osborne (1954) also found an increase in the γ-globulin of piglets after colostral feeding.

Norbring (1957) studied the change in the electrophoretic pattern and antibody titre in porcine colostrum during the first days of lactation. He observed a marked decrease in slowly migrating globulins during the first two or three days after parturition. He also found that there was a high concentration of Salmonella Paratyphi A H-agglutinins in the colostrum samples of farrowing sows immunized during pregnancy with paratyphoid A vaccine. By starch electrophoresis he showed that the antibodies were localized in the immune globulins. Nordbring and Olsson (1957) made similar studies on the sera of newborn pigs before and after the ingestion of porcine colostrum. They found that the cord blood had an extremely low concentration of γ-globulin and albumin; the dominating factor was α_2-globulin. In the course of few hours after the ingestion of antibody-containing colostrum there was a marked increase in the amount of globulins of slow electrophoretic mobility accompanied by a rapid appearance of antibodies in the piglets' sera. They also found that this passively acquired γ-globulin decreased steadily

during the first weeks of life, reaching a minimum value at about four weeks of age. After this age the γ-globulin level remained constant or slightly increased due to the autogenous synthesis of γ-globulin. There was also a continuous decrease in the antibody titre during this period.

By feeding known amounts of immune globulins from the colostrum Nordbring and Olsson (1958a) calculated the relative amounts of these globulins entering the blood of the newborn pig from the intestinal lumen. Newborn pigs were fed by stomach tube with immune porcine colostrum containing Salm. Paratyphi H agglutinins at birth and at 24, 36, 48 and 72 hours of age. During the period of deprivation of colostrum the piglets received fluid orally and parenterally. The authors found that when feeding of immune colostrum was started immediately after birth the relative increase in concentration of the slowly migrating globulins corresponded approximately to 17 per cent. of the amount of the immune globulin given. The amount of the agglutinins absorbed at birth varied from 4 to 21 per cent. of the amount given. When feeding was started at 72 hours of age the relative increase in the globulin was approximately 4 per cent. and the amount of agglutinins absorbed was from 0.2 to 3 per cent. of the amount given. These authors also found that when trypsin inhibitor obtained from bovine colostrum was added to the porcine colostrum before feeding to some of the pigs used in the above experiment there was

a high relative increase in the slowly migrating globulins in the piglets' sera. They attributed this increase to a protective effect of the inhibitor against trytic digestion of the proteins.

Nordbring and Olsson (1958b) investigated the absorption of the protein components and the antibodies present in porcine serum by the newborn pig after oral administration at birth, 26 and 4C hours of age. The paper electrophoretic patterns and the agglutinin titres of the serum administered orally, of the cord and of the piglets' sera after feeding were examined. When the immune serum was given at birth, antibodies appeared in all the sera of the piglets. The antibodies absorbed amounted to 4 - 15 per cent. of the amount administered. When the feeding of the immune serum was started at 40 hours of age no measurable absorption of antibodies occurred. The relative increase of various fractions in the piglets' sera after the oral administration of the porcine serum was also calculated. The highest relative increase was in the γ-globulin fraction when the serum was given at birth; in this case concentration reached a level corresponding to absorption of about 1C per cent. of the amount given, whilst the corresponding values for β-globulin and albumin were only 5 and 4 per cent. respectively. When bovine trypsin inhibitor was given with the serum there was a higher relative increase in the electrophoretic fractions in the

piglets' sera.

Barrick et al. (1954) however, found that when porcine γ-globulin was administered orally there was no rise in the γ-globulin levels of newborn piglets.

Rutgvist (1958) made paper electrophoretic examinations of sera collected from pig foetuses and newborns before and after suckling. He found that in the sera of foetuses at 2½ and 3½ months of gestation and in those of newborn pigs three fractions were present, albumin and α- and β-globulin. No γ-globulin was present in these sera, but large amounts appeared shortly after nursing.

Speer, Brown, Quinn and Carton (1959) found that antibody absorption by baby pigs declined significantly after the first 24 hours after birth. They also found that the ability of the young pig to absorb antibody fell by 50 per cent. every three hours.

Sterzl, Kostka, Mandel, Riha and Holub (1960) by paper and boundary electrophoresis showed that no γ-globulin was present in colostrum-deprived pigs up to three weeks of age. However when the newborn pig's serum was concentrated 50 to 100 times by alcohol fractionation and on diethylaminoethyl (DEAE) cellulose, some protein was detected by electrophoresis in the γ-globulin fraction. The amount detected corresponded to 10-40 µgs. γ-globulin per ml. of original piglet's serum. These authors did

not find antibodies in newborn piglets' sera born to sows that had very high titres of antibody to Brucella suis. Antibodies were not found in the piglets' sera even after concentration of the serum to one seventh the original volume. They concluded that γ-globulin might be synthesized in newborn animals.

Myers and Segre (1963), using haemagglutination tests for the determination of the antibody titres, reported transplacental transfer of diphtheria and tetanus antitoxin in colostrum-deprived pigs. Their results showed the presence of diphtheria and tetanus antibodies in the 10-times concentrated γ-globulin fraction of pooled sera of newborn pigs born to actively and passively immunized sows. However, no antibodies could be detected in whole unconcentrated pooled sera of colostrum-deprived newborn pigs of actively and passively immunized mothers.

The formation of antibodies by newborn pigs was studied by Segre and Kaeberle (1962 a,b). They found that colostrum-deprived baby pigs of three weeks of age were poorer antibody producers than pigs of the same age that were fed with colostrum. They explained their findings on the basis of the natural selection hypothesis of antibody formation proposed by Jerne who postulated that antibodies are formed independently of stimulation by antigen and are necessary for the formation of antigen-stimulated antibodies. However, these authors found that larger amounts of

antibodies acquired passively through the colostrum inhibited the formation of antibodies in piglets given injections of toxoid. These authors also found that γ-globulin was present in the serum of newborn pigs deprived of colostrum. This was accomplished by allowing rabbit anti-swine γ-globulin and blood of colostrum-deprived pigs to diffuse against one another in agar gels.

Wellmann, Liebke and Engel (1962) found that the swine erysipelas antibody content of colostrum was usually higher than that of the maternal blood serum. However, in some sows the reverse was true (Wellmann, Schwitzer and Liebke, 1961). Wellmann et al. found that the antibodies present in the milk started to decrease within the first 24 hours after parturition. When the initial titre in the milk was high, the antibodies could be demonstrated even at eight weeks after parturition. The absorption of these antibodies by piglets was found to be greatest during the first 24 hours of postnatal life and decreased considerably on the second day. Absorption of antibodies from the colostrum by the piglets was demonstrated even at 120 hours after birth (Wellmann and Engel, 1963).

Lecce, Morgan and Matrone (1964), having found that the absorption by neonatal piglets of polyvinylpyrrolidone (PVP) given by mouth ceased earlier in piglets given pig or cow colostrum than in starved piglets, attempted to find out what the substance in colostrum producing this

effect (which they called "closure") was. They found that "closure" to PVP and cow γ_2-globulin was not produced by fat or protein as it is present in colostrum, but is associated with non-fat non-protein dialysable fraction of colostrum.

Olsson (1959a,b), who examined cord blood and serum of colostrum-fed pigs by electrophoresis and serological methods showed that newborn piglets absorbed agglutinins from cow and sow colostrum and horse serum (given by mouth) equally readily.

Payne and Marsh (1962) also studies the selectivity of the gut of newborn pigs. Porcine, bovine, ovine and human colostrum were tube-fed to colostrum-deprived pigs obtained by hysterectomy. Gamma-globulin was absorbed from all these materials, and its presence in the piglets sera was demonstrated by sensitive methods like gel electrophoresis, immuno-diffusion and fluorescent microscopy. Equine, bovine, human and porcine γ-globulins were tagged with fluorescent dye and were injected into 5.0 cm. long intestinal segments of six-hour-old pigs. These segments were then examined with the fluorescent microscope. The results showed that all of these globulins were absorbed by the gut. The authors concluded that the newborn piglet's gut was not selective in absorption of heterologous globulins. The last experiment was repeated with porcine and bovine serum β-globulins and bovine α-globulin; all

were absorbed by the intestinal segments.

Payne and Marsh (1962) also found that the absorption of γ-globulin obtained from sow's colostrum and tagged with fluorescein isothiocyanate ceased 12 hours postnatally in pigs that were allowed to suck normally or were fed on modified cow's milk. In pigs starved or given water there was a marked absorption of γ-globulin for 106 hours postnatally.

Locke, Segre and Myers (1964) found that colostrum-deprived newborn pigs absorbed low molecular weight (6.6S) diphtheria and tetanus antibodies efficiently, whereas high molecular weight (18S) antibodies were absorbed poorly or not at all.

SUMMARY

1. Piglets acquire their passive immunity through the ingestion of colostrum and not by way of the placenta.
2. No antibodies are present in the sera of piglets born to immune sows.
3. Antibodies appear in the blood of newborn pigs after the ingestion of antibody-containing colostrum.
4. Antibodies may be absorbed by piglets even on the fifth day after birth.
5. Low concentrations of γ- and large amounts of α2-globulins are present in the piglets' serum before colostral ingestion.

6. Marked increase in γ-globulin of slow electrophoretic mobility accompanied by rapid appearance of antibodies occurs after colostral ingestion by baby pigs.

7. Gamma-globulin absorption by piglets may be enhanced by the addition of trypsin inhibitor.

8. Antibodies present in the colostrum are localized in the γ-globulin fraction and may be of higher titre than those of the maternal serum.

9. Piglets can absorb antibodies from immune porcine serum administered orally.

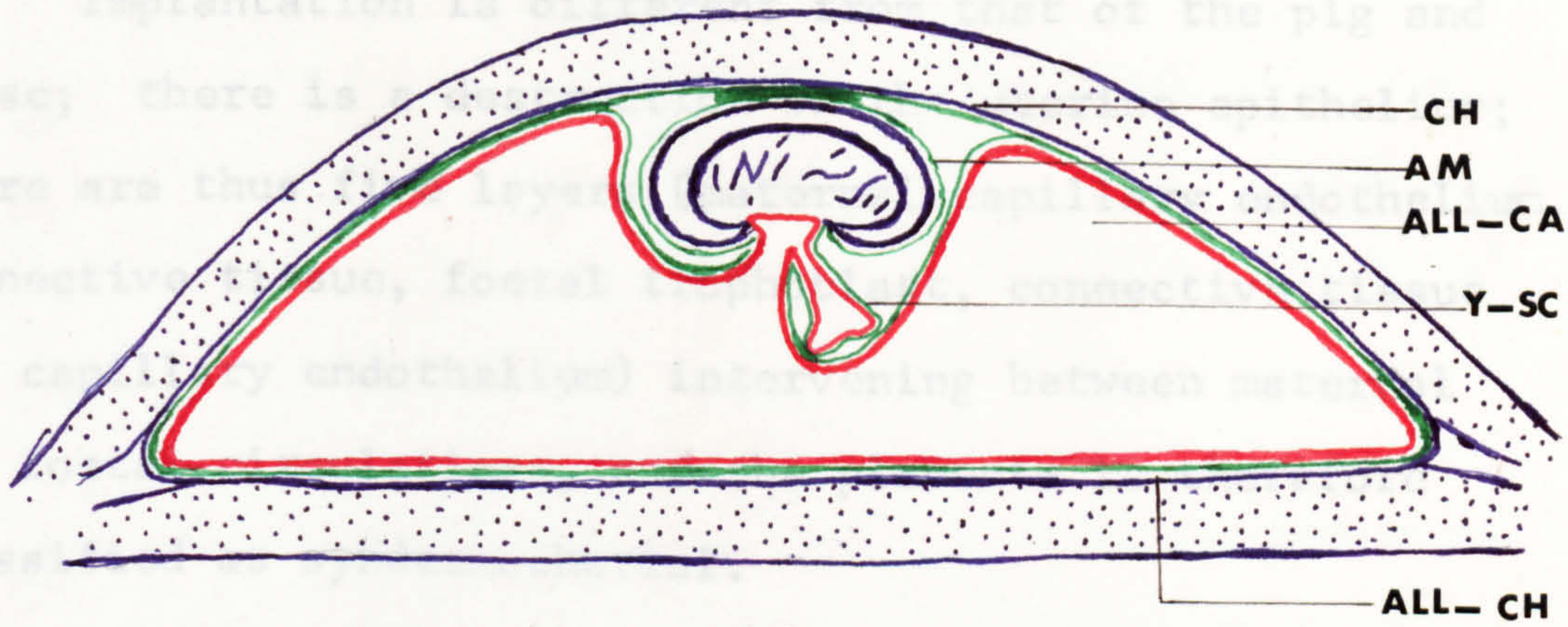
10. The passive immunity acquired by the piglets from the colostrum may be lost by the end of the third month of postnatal life.

11. The newborn piglets' gut, like that of the newborn calf and unlike the yolk-sac splanchnopleur of the rabbit, is non-selective in nature.

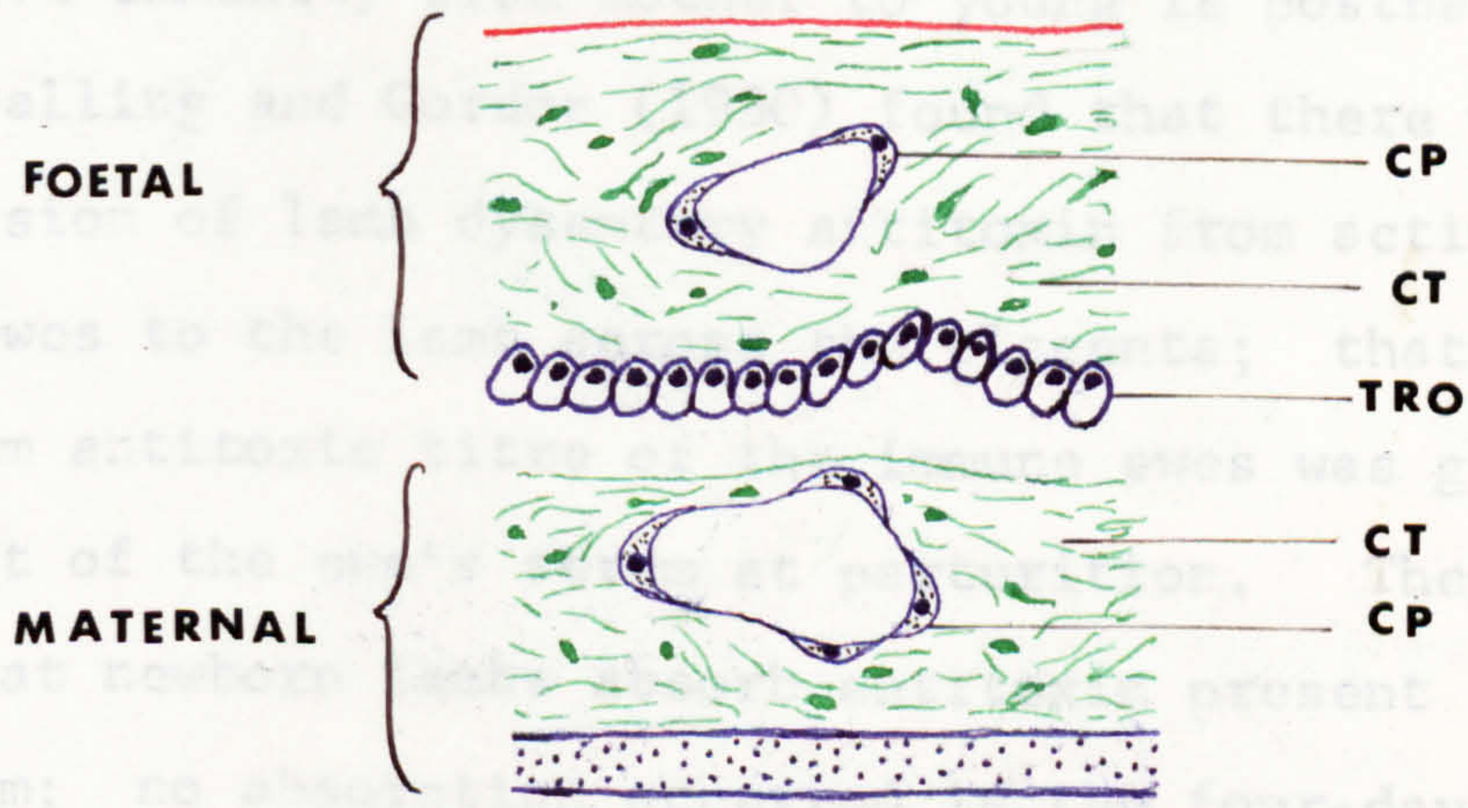
THE SHEEP

Foetal membranes and placentation:

In sheep the embryonic development and arrangement of foetal membranes are like those in the pig, with the exception that the allantoic vesicle entirely obliterates the exocoelic cavity (Fig. 5). The membranes that are exposed to the uterine lumen are therefore, in turn, the bilaminar omphalopleur, chorion and bilaminar omphalopleur, allantochorion.



(a)



(b)

syndesmochorial placenta

Figure 5

Embryonic membranes and placenta of sheep

AM, Amnion; ALL-CA, allantoic cavity; ALL-CH, allanto-chorion; CH, chorion; CP, capillary; CT, connective tissue; TRO, trophoblast; Y-Sc, yolk-sac. (a is after Mossman, 1937; b is after Amoroso, 1952).

Implantation is different from that of the pig and horse; there is a destruction of the uterine epithelium; there are thus five layers (maternal capillary endothelium, connective tissue, foetal trophoblast, connective tissue and capillary endothelium) intervening between maternal and foetal circulations, and the placenta is therefore classified as syndesmochorial.

Transfer of immunity:

In sheep, as in the horse and the pig, the transfer of passive immunity from mother to young is postnatal. Mason, Dalling and Gordon (1930) found that there was no transmission of lamb dysentery antitoxin from actively immune ewes to the lamb across the placenta; that the colostrum antitoxic titre of the immune ewes was greater than that of the ewe's serum at parturition. They also found that newborn lambs absorb antitoxin present in the colostrum; no absorption occurred in the four-day-old lamb. When heterologous (horse) antitetanus serum was given, antitoxin was detectable in the lamb's sera half an hour later. When horse antidiphtheria serum was injected intravenously into the ewe and this antitoxin, present in the colostrum, was fed to the newborn lamb, diphtheria antitoxin was detected in the lamb's serum after half an hour. In other words when the heterologous antitoxin was present in the colostrum, it was absorbed by the newborn lamb.

Earle (1935) found that the blood of the newborn lamb contained no significant quantity of euglobin and very small quantity of pseudoglobulin 1. The quantities of these globulins increased in 24 hours after colostral feeding.

Schneider and Szathmary (1939a) immunized pregnant sheep against Salm typhi and diphtheria toxoid and found that the antibacterial and antitoxic antibodies were transferred to the foetus to very low titres only; transfer of these antibodies was mainly through the colostrum.

Pedersen (1945) by fractionation experiments with ammonium sulphate showed that foetal sheep serum contained negligible amounts of normal globulin. He also showed by ultracentrifugation that fetuin predominates in the foetal serum.

Smith and Holm (1948) studied the electrophoretic patterns of the serum of the newborn lamb before and after the ingestion of colostrum. They found that slow-moving globulins appeared in the serum only after suckling.

Charlwood and Thomson (1948) also by electrophoretic analysis found that lamb serum at birth was almost entirely lacking in γ-globulin. Twenty-four hours later, after suckling, there was a large increase in γ-globulin.

McCarthy and McDougall (1949) found that in ewes immunized during the latter half of pregnancy with Salm. typhi H-antigen, the immune globulin appeared in

colostrum over a short period before lambing. They also found that in lambs that were suckled normally the serum globulin, mainly γ-globulin, and agglutinins increased rapidly, owing to the absorption of immune globulin. The serum albumin, on the other hand, showed a transient fall soon after birth, then rose to adult values at five weeks. They also found that there was no absorption of globulin in lambs 48 or more hours old.

Hill (1956) investigated the cause of the cessation of antibody absorption by the lamb. He found that the immune globulins cease to be absorbed because of increasing activity of the digestive enzymes in addition to changes in the permeability of the small intestine. He found that the number of parietal cells in the abomasum of the foetal lamb was very low until term; their number increased rapidly during the first 48 hours of postnatal life. Hill also showed that the abomasal secretion was deficient in hydrochloric acid at birth; it increased during the first three days of life. The pH of the abomasal content changed from 6.0 and 7.0 at birth to 3.0 - 4.0 after 36 hours.

Hill and Hardy (1956) made histological and histochemical studies on the intestinal epithelium of young lambs and kids shortly after colostral feeding. Mucoproteins or glycoproteins were found to be present in the lumen of the gut, in the epithelial cells and in the

lacteals. They suggested that colostral globulin became associated with mucus secreted by the digestive tract before being absorbed by the cells.

Cumming and Bellville (1963) studied the antibody content of maternal and foetal blood and amniotic fluid before and after immunization of the ewe with Salmonella Pullorum in the last trimester of pregnancy. They found that no transfer of antibodies from mother to foetus could be demonstrated in utero.

Professor Oakley tells me that some unpublished work by Bullen, Oakley, Batty and Scarisbrick suggests that the intestine of the lamb absorbs γ-globulin unselectively for the first sixteen to twenty-four hours of postnatal life.

SUMMARY

1. The foetal lamb, like the piglet, does not acquire its immunity in utero.

2. The foetal lamb is born with almost no γ-globulin in its serum, but possesses large amounts of fetuin.

3. After suckling slow moving γ-globulins appear in the lamb serum.

4. Immune globulins may accumulate in the colostrum shortly before lambing.

5. No absorption of immune globulins occurs in lambs more than forty-eight hours old.

6. The cessation of absorption of globulins by the lamb gut may be due to increase in the number of enzyme-secreting

cells and change of pH.

7. The intestine of newborn lamb absorbs γ-globulin unselectively for the first sixteen to twenty-four hours of postnatal life.

THE COW

Foetal membranes and placentation (largely based on Amoroso in Marshall's physiology of reproduction, 1952)

In cow the foetal membranes and placentation are similar to those in the sheep (Fig 5). Therefore the membranes that are exposed to the uterine lumen are in turn, the bilaminar omphalopleur, chorion and biliminar omphalopleur, allantochorion. The placenta, like that of sheep, is syndesmochorial with five layers intervening between the foetal and maternal circulations.

Transfer of immunity.

In cattle practically all the evidence favours the view that the transfer of immunity is postnatal and that colostrum plays a primary role in this transfer. It was found by Howe (1921) that the serum of a newborn calf did not contain proteins precipitable by 17.4 per cent. sodium sulphate before the ingestion of colostrum. He suggested that eugolbulin and pseudoglobulin 1 were absent from the blood of the newborn calf. These globulins appeared in large amounts in the serum of calves that had received colostrum.

Little and Orcutt (1922) found that no agglutinins

were present in the blood of calves born of cows naturally immune to Brucella abortus. These antibodies were present in the serum after colostral feeding. Orcutt and Howe (1922) confirmed the above finding and found an association between the increase of total globulin and the rise in the agglutinin titre in the blood of the newborn after suckling.

During their investigation of bovine infectious abortion, McAlpine and Rettger (1925) investigated the effect of ingestion of colostrum on the appearance of Br. abortus complement-fixing and agglutinating antibodies in the serum of newborn calves. When cows that possessed these antibodies calved, their calves had at birth no such antibodies in their sera. Agglutinins and complement-fixing antibodies appeared a short time after the ingestion of immune colostrum when calves were permitted to suck within the first twenty-four hours of life. They also found that the titre of the ingested colostrum determined the time within which these antibodies disappeared from the calves' serum. The serum of calves fed on high titre colostrum remained positive longer than that of calves fed on low titre colostrum. These passively transferred antibodies disappeared before the calf was six months old. Thorpe and Graham (1933) also found that the time taken for antibodies passively acquired from colostrum to disappear from the calf circulation was six months or less.

Mason, Dalling and Gordon (1930) showed that a calf

born to a cow immune to diphtheria toxin did not have detectable antitoxin in its serum. Twenty-four hours after suckling, the calf had antitoxin in its serum. They also found that heterologous antitoxic sera were absorbed by the newborn calf. When sheep and horse antisera were given to the calf by mouth twelve hours after birth, both were detected in the calf's serum twenty-four hours later. The elimination of horse antiserum from the calf circulation was faster than the elimination of sheep antiserum.

Smith (1930) showed that the antibody concentration in normal cow serum was less than that in the colostrum of the same animal. When homologous antiserum was given by mouth to two calves aged two days and five hours and eighteen days, no agglutinins were demonstrated in their blood after feeding. Smith and Little (1922, 1930) showed that colostrum was important for the protection of newborn calves against Bacterium coli infection; the feeding of newborn calves with immune cow serum against B. coli gave certain protection against death from B.coli infections.

Minett (1937) investigated the concentration of Staphylococcus α-antitoxin in colostrum compared with that of the cow serum. His results showed that the α-antitoxin titre in colostrum was at least equal to, and often exceeded, that in the serum, whilst the concentration of the antitoxin in the milk was 1/80 to 1/40 of that in the blood.

Kerr and Robertson (1943, 1946) showed that calves born of cows actively immunized with Trichomonas foetus before or during pregnancy were born without agglutinins in their blood. An agglutinin titre approximating that of the colostral whey was rapidly acquired by the calves after the ingestion of colostrum. The highest antibody titre was reached twelve to sixteen hours after the ingestion of colostrum. No absorption of antibody occurred if the calf was fed on boiled milk twenty-four hours before colostral feeding. Kerr and Robertson (1954) found that these passively acquired antibodies disappeared from the calf serum at a logarithmic rate with a half life ranging from fourteen to twenty days. The logarithmic disappearance of the passively acquired antibodies was later confirmed by Pierce (1955b).

McDiarmid (1946) also found that calves born to cows vaccinated against Br. abortus had no agglutinin in their blood. The agglutinins appeared after the ingestion of colostrum containing antibody; their concentration reached its maximum in twenty-four hours and then fell at a logarithmic rate. These agglutinins persisted in the calf's blood over a period that depended on the titre of the colostrum; the higher the titre, the longer the period of persistence of antibodies.

Williams (1961) found that calves born to rabies-vaccinated cows did not show neutralizing antibodies in

their sera. Colostral antibodies passed to the calf in appreciable quantity. Such antibodies persisted for about twelve to fourteen weeks after birth. Williams (1961) also found that when calves possessed negligible amounts of antibodies they responded to rabies vaccination with a tenfold rise in the serum neutralizing antibody titre, whereas colostrum-fed calves with appreciable quantities of antibody in their sera showed no antibody rise in response to vaccination. As far as I can see from Johnson and Pierce's (1959) data, the failure of calves given colostrum to produce antibody was not due to their incapacity to synthesize γ-globulin.

Graves (1963) found in eight of the ten animals studied that the antibody titre of the colostrum of cows vaccinated against foot-and-mouth disease was higher than that of the serum, but the difference was not significant. There was no transfer of the virus-neutralizing antibodies from cow to calf in utero. The transfer of antibodies was by colostrum only. He also found that the feeding of calves with skimmed milk thirty minutes after birth and three hours before colostral feeding blocked the transfer of neutralizing antibody.

Schechtman and Abraham (1958) and Kulangara and Schechtman (1963) found that even when as a result of intravenous injection of human albumin, the human albumin in the pregnant cows circulation reached a

concentration at which in pregnant rabbits, it would have passed into the foetal circulation, no albumin was transmitted to the foetus.

The importance of colostrum in the transfer of immunity was also studied by physico-chemical techniques as well as immunological and serological methods. Electrophoretic analysis and ultracentrifugation were used by many investigators. Jameson, Alvarez-Tostado and Sorter (1942) studied the composition of newborn calf serum by electrophoresis before and after the ingestion of colostrum. They found that γ-globulin was absent in the serum before the ingestion of colostrum, but appeared rapidly during the suckling period.

San Clemente and Huddleson (1943) also studied the electrophoretic patterns of newborn calves' sera. They found that the serum of a newborn calf had an extremely high concentration of α-globulin, but that γ-globulin was present in very low concentration or was absent. Four hours after the ingestion of colostrum by a normal calf the γ-peak rose and accounted for about fifteen per cent. of the total protein. Brucella abortus agglutinins were also absorbed from the colostrum. By the end of two weeks all protein components reached the normal level usually found in young heifers.

Smith (1946) found that the immune activity* of bovine plasma was present in two well defined components, the Y and T; these components differed in the electrophoretic mobility and their iso-electric points. The colostrum immune globulin was similar to the T component of plasma in its iso-electric point and electrophoretic mobility, but they differed in their amino acid composition and ultra-violet absorption spectra. Smith and Holm (1948) found that the newborn calf serum did not contain Y nor T globulin. After the ingestion of colostrum a component appeared in the newborn calf serum that had an electrophoretic mobility identical with that of the globulin that was present in the colostrum. Antibodies that were present in the colostrum also appeared in the calf serum after colostral feeding.

Hansen and Philips (1947) by electrophoretic analysis studied the blood serum proteins of colostrum-deprived calves and of calves that had received colostrum. They found that γ-globulin appeared after the ingestion of colostrum during the first twenty-four hours of life. No increase in serum proteins occurred if calves over twenty-four hours old were fed on colostrum.

---

* This "immune activity" was not defined; it was said to be dealt with in detail in a paper by Holm which as far as I could discover remained unpublished.

Pierce (1955) compared the electrophoretic patterns of serum proteins of calves from birth to weaning with those of colostral whey. He found that in calf serum before colostral feeding albumin and two other components with mobilities similar to those of α-and β- globulins of adult serum were present. Another component with a mobility similar to that of γ_1-globulin or fibrinogen was present. This component, which formed 1.4 per cent. of the total serum protein, represented the γ-globulin which, the author thought, might be autogenous γ-globulin or globulin passively acquired <u>in utero</u>. In colostrum-fed and colostrum-deprived calves antogenous γ-globulin was evident shortly after birth. By the tenth day after birth the γ_1 and γ_2 components appeared, and by the thirtieth day the γ_3 components were detected. By the thirtieth day minimum α-globulin values were reached in both colostrum-fed and colostrum-deprived groups. A marked but transient increase of β-globulin occurred when the α-globulins were at their lowest values. The mobilities of the electrophoretic components of calf and adult sera did not show any significant difference.

Graves (1963) found by immunoelectrophoretic study that no γ-globulin was present in the serum of calves at birth, but it was detectable two hours after the ingestion of colostrum.

Ultracentrifugal studies were also made on the

newborn calf serum. Pedersen (1944) made ultracentrifugal studies on fractions separated by ammonium sulphate precipitation from serum from calves not more than two weeks old. He found that large amounts of a globulin with a sedimentation constant of 3S were present. "Fetuin" (the name proposed for the new protein) appeared as an extra peak close to that of albumin in the ultracentrifugal runs (Pedersen, 1945). This globulin, "fetuin", was precipitable between 0.37 and 0.45 saturation with ammonium sulphate; its iso-electric point was pH 3.5 (Pedersen, 1947) and it had a molecular weight of about 50,000. It was later found by Deutsch (1954) that fetuin was a mucoprotein of greater lability than the mucoproteins obtained from other systems. Pierce (1955) found that in calves deprived of colostrum the α-globulin, fetuin increased immediately after birth and then fell, whilst in colostrum-fed calves it declined shortly after birth.

Johnson and Pierce (1959) made an ultracentrifugal and electrophoretic studies of the proteins in the maternal colostrum and serum from calves fed on or deprived of colostrum. A correlation between the two types of results was made by preparing certain fractions electrophoretically and examining them in the ultracentrifuge. Johnson and Pierce showed by electrophoresis that in precolostral calf sera there was

a very low concentration (about 2.0 per cent. of the total protein) of the usual adult γ-globulin. They confirmed this result by showing by ultracentrifugation the almost complete absence of globulin with a sedimentation constant $S^{o}_{20} \simeq 6.5 - 7.0$ S. In postcolostral calf sera there was evidence, in both electrophoretic and ultracentrifuge analysis, of the absorption of immune lactoglobulin from the maternal colostrum. A rise in the concentration of total serum protein was found in the calf's serum after colostral feeding. About 50 per cent. of this rise in the serum protein of the colostrum-fed calf was immune lactoglobulin of sedimentation constant 6-6.5 S. These authors also found that in colostrum-deprived calves under three weeks of age, there was autogenous γ-globulin formation, which increased with time. This autogenous γ-globulin had a molecular weight, shape and general properties similar to those of the γ-globulin found in adult sera.

The route of absorption of colostrum globulin was investigated by Comline, Roberts and Titchen (1951a). They found that Br. abortus agglutinins appeared in the lumph after the introduction of antibody-containing colostrum whey into the small intestine of newborn calves. Lymph was collected from the intestinal lymphatic trunk and/or the thoracic ducts and blood from the jugular vein, and their golublin contents were estimated by agglutination

tests and nitrogen estimation after precipitation with sodium sulphite. It was shown (Comline, Roberts and Titchen, 1951a) that when the thoracic duct was cannulated, agglutinins appeared in the lymph, but not in the blood, one to two hours after the introduction of whey in the duodenum of young calves six to twenty-seven hours of age. When whey was introduced into the small intestine 63-65 hours after birth agglutinins were either absent from the thoracic duct lymph or present in very low concentration one to two hours after introduction. When the intestinal lymphatic trunk was cannulated in addition to the thoracic duct globulins were confined to the intestinal lymph. When whey was introduced into the abomasum and the large intestine, after separation of the small intestine by ligatures, no colostrum proteins were present in the lymph or blood. The authors concluded that in young calves colostrum proteins are absorbed from the small intestine and then carried in the lymph to the peripheral blood and do not enter the portal circulation in appreciable amounts. Comline, Roberts and Titchen (1951b) studied the histological changes in the cipthelium of the small intestine during protein absorption. Pieces of the small intestine were taken from calves three to twenty-four hours old that were being fed on colostrum, and fixed and stained with haematoxylin, azocarmine, eosin or orange G. The colostral proteins appeared to pass through the cells

of the intestinal epithelium. Balfour and Comline (1962) later confirmed the above findings.

The selectivity of the gut of the young calf for colostrum and serum proteins was investigated by Bangham, Ingram, Roy, Shillam and Terry (1958) using tracer and electrophoretic techniques. They found that the gut of the new-born calf showed no selectivity towards labelled adult bovine serum and colostral proteins. The proteins present in the serum and colostrum were absorbed with equal facility. Electrophoretic fractions of calf serum samples taken three, six and twenty hours after feeding with tracer-labelled whole serum and examined for radioactivity showed that serum albumin and β-globulin disappeared from the circulation faster than γ-globulin.

Pierce and Feinstein (1965) found that the differences between cow and calf serum proteins are not due to selection of particular colostrum components by the calf intestinal epithelium, but to selection by the mammary gland during the production of colostrum. They found that the newly-born calf's intestine showed no selectivity; it absorbed equally readily immune globulins showing three different electrophoretic mobilities but that "the mammary gland showed a highly selective preference for, and hence ability to concentrate in, colostrum, the electrophoretically fastest serum immune globulin." They also found that colostral immune lacto-globulins were qualitatively similar to the immune globulin present in bovine serum.

It is interesting to note here that cattle milk proteins are preformed outside the mammary gland before they are incorporated in the milk. In other words they are transmitted via the circulation to the mammary gland and not degraded and resynthesized in the gland (Larson and Gillespie, 1957).

SUMMARY

1. In cattle there is no transfer of immunity from mother to young *in utero*.
2. Calves are born with no γ-globulins of sedimentation constant 7S in their blood; at birth they possess large amounts of γ-globulin of sedimentation constant 3S.
3. There is a rise in the total globulin of newborn calves' sera after the ingestion of colostrum. This rise in total globulin is associated with a rise in the antibody titre of their sera.
4. Thecolostrum antibody titre may equal or even exceed that of the serum of the cow. The immune globulins have similar electrophoretic mobilities and are qualitatively similar to those in bovine serum and they seem to be preformed outside the mammary gland before being incorporated in the milk.
5. The antibodies present in the colostrum were absorbed by the newborn calf within the first twenty-four hours of postnatal life. The persistence of the passively acquired

antibodies in the newborn calf's circulation depends on the titre and amount of the ingested colostrum. The antibodies acquired by the newborn calf from the colostrum decline at a legarithmic rate, disappearing during a period of six months or less.

6. Not only homologous globulins can be ingested and absorbed by the newborn calf, but also heterologous proteins.
7. The proteins ingested by the newborn calf are absorbed by the intestinal mucosa with equal facilities.
8. Unlike the yolk-sac splanchnopleur of foetal rabbits, the intestinal epithelium of newborn calves shows no selectivity in its absorption of globulins.
9. Selectivity of proteins seems to occur within the mammary glands during the production of colostrum.
10. The colostral proteins absorbed by the intestine of the newborn calf reach the peripheral circulation via the intestinal lymphphatic trunk.

THE GOAT

Foetal membranes and placentation in the goat are similar to those in the sheep and cow.

Transfer of immunity:

Like calves and lambs, kids acquire little if any immunity *in utero*. Famulener (1912) immunized goats with sheep red blood cells and found that little if any haemolysin were acquired by the foetus. He also found

that the colostral haemolysin titre was higher than that in the maternal serum and that the passive immunization of the newborn kid was chiefly colostral. In one experiment Famulener fed the newborn kid on homologous haemolytic serum and found that the serum was absorbed in considerable amounts. He also found that there was no transfer of antibodies from the colostrum in older kids. He concluded that the placenta plays a minor or negligible role in the transfer of haemolysins to the foetal kid.

Reymann (1920) found that no agglutinins were present in the serum of kids born to goats having normal agglutinins towards Escherichia coli and Salm. typhi. The colostral agglutinin titre was higher than that of the maternal serum. He also found that the agglutinin in the kid's serum reached its maximum value at about eleven hours after birth.

Earle (1935) found that euglobulin was present in low concentration in the newborn kid's serum and pseudoglobulin 1 was present in rather larger concentration. He also found that after colostral feedint these two proteins increased in quantity.

Ultracentrifugal studies as well as protein estimations were made by Deutsch and Smith (1957). They found that all of the colostrum whey proteins were absorbed during the first twenty-four hours of postnatal life in goats and a new component of sedimentation constant

6.5S appeared after colostral ingestion.

Askonas, Campbell, Humphrey and Work (1954) found that in the goat, as in the rabbit, the immune globulins pass unchanged from the blood stream into the mammary gland and are not degraded and resynthesized in the glands themselves.

According to McGirr (1947) antibody absorption is supposed to cease in kids at some time earlier than four days. I have not found any papers about the time of cessation of antibody absorption in kids.

SUMMARY

1. Kids acquire little if any immunity in utero; their passive immunization is chiefly colostral.

2. Colostral antibody titre may exceed that of the maternal serum.

3. Antibodies can be absorbed not only from the colostrum but also from serum given by mouth.

4. The immune globulins are not degraded and resynthesized in the mammary gland; they pass unchanged from the blood serum and are incorporated in the milk.

5. Antibody absorption by the gut of the kid seems to cease before the kid is 4 days old.

PRE- AND POSTNATAL TRANSFER OF IMMUNITY

Ehrlich's (1892) classical "exchange or wet-nurse" experiments on mice and his discovery of transfer of immunity via the milk were the start of many researches in the field of immunology. Young rats and mice acquire their passive immunity before and after birth; the latter being the main route (Culbertson, 1938, 1940).

RAT AND MOUSE

Foetal membranes and placentation

The blastocyst of the rat and the mouse consists of a layer of trophoblast covering an inner embryonal cell mass one edge of which differentiates into a thin layer of endoderm. In the inner cell mass a cavity, the proamniotic cavity, appears. The cells that form its roof thicken to form the "Träger" (Fig 6a). These cells later grow considerably and thereby push the floor of the inner cell mass and the endoderm forwards; the endoderm grows to cover the mass so formed. The main mass of ectoderm is now covered by endoderm; an inversion of germ layers has already occurred. Later the roof of the proamniotic cavity breaks down and the cavity becomes continuous with that due to the central breakdown of the "Träger" (Fig 6b).

By the seventh day the endoderm has extended to form a thin layer lining the trophoblast; a bilaminar omphalopleur is thus formed enclosing the yolk-sac cavity.

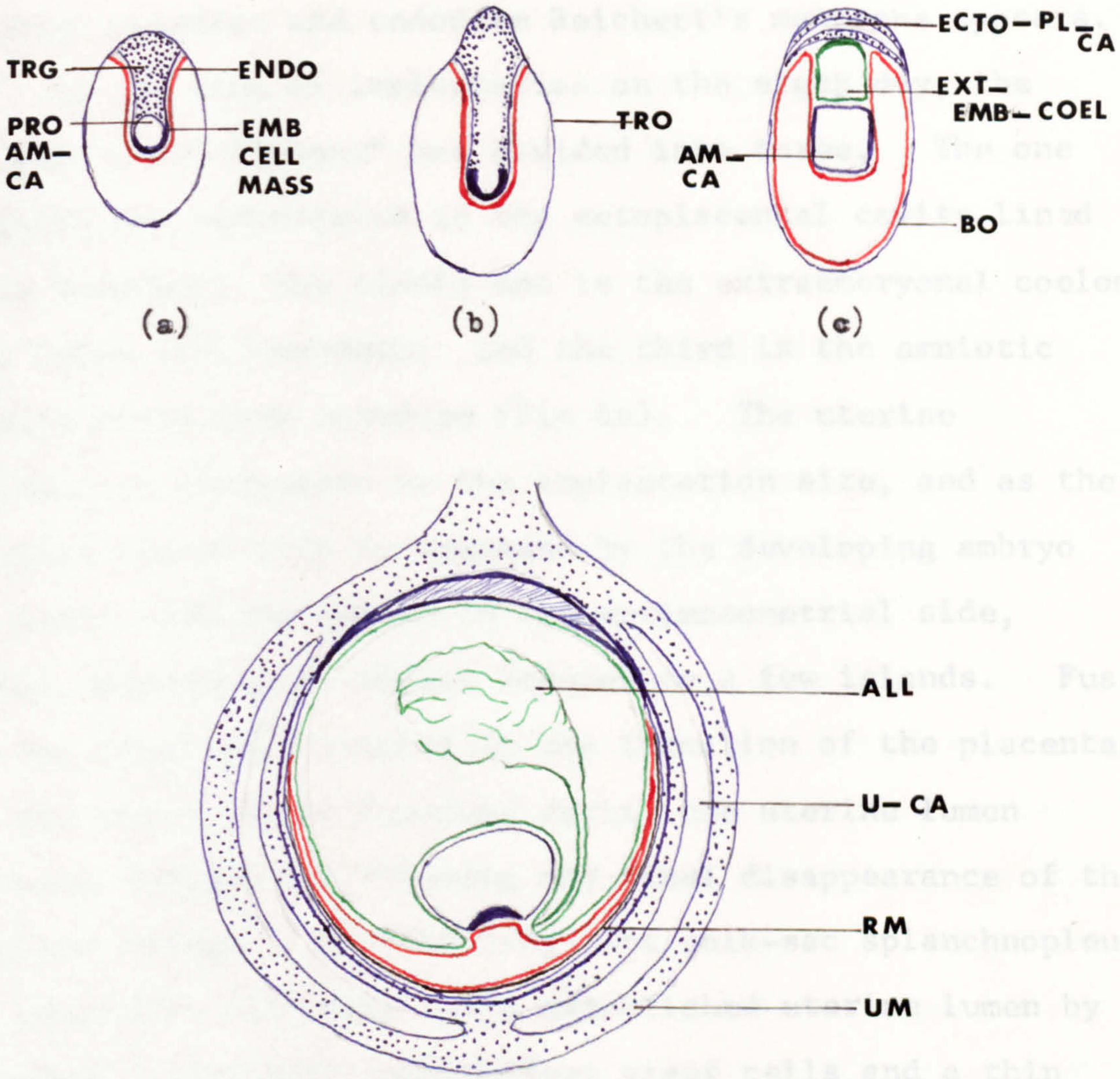


Figure 6

Blastocyst and early development of embryonic membrane of rat and mouse;

AM-CA, amniotic cavity; ECTO-PL-CA, ectoplacental cavity; EMB-CELL-MASS, embryonic cell mass; EXT-EMB-COEL, extra-embryonal coelom; PRO-AM-CA, proamniotic cavity; RM, Reichert's membrane; TRG, träger; U-CA, uterine cavity. For ALL, BO, ENDO, TRO and UM see figure 1. (a) is after Duval, 1892; b is after Sansom and Hill, 1931; c is after Amoroso, 1952 and d is after Mossman, 1937)

Between ectoderm and endoderm Reichert's membrane appears.

By the time of implantation on the eigth day, the cavity in the "Träger" has divided into three. The one nearest the mesometrium is the ectoplacental cavity lined with ectoderm; the middle one is the extraembryonal coelom now lined with mesoderm; and the third is the amniotic cavity lined with ectoderm (Fig 6c). The uterine epithelium disappears at the implantation site, and as the uterine tissue here is expanded by the developing ambryo it fuses with the mucosa on the antimesometrial side, whose epithelium is slowly reduced to a few islands. Fusion, on the eigth day, results in the formation of the placenta. At the floor of the decidual cavity the uterine lumen reforms through the thinning and final disappearance of the uterine tissue. At this stage the yolk-sac splanchnopleur is separated from this newly established uterine lumen by Reichert's membrane, mononuclear giant cells and a thin muscular layer. The giant cells become greatly reduced later. The layer separating the yolk-sac splanchnopleur from the uterine cavity ruptures between the fifteenth and sixteenth day exposing the yolk-sac vascular endoderm to the uterine lumen.

The allantois grows out from the posterior end of the embryo. It consists of a solid mass of mesoderm with no endodermal cavity. It gradually grows into the extra-embryonal coelom. On about the eigth day it fuses with

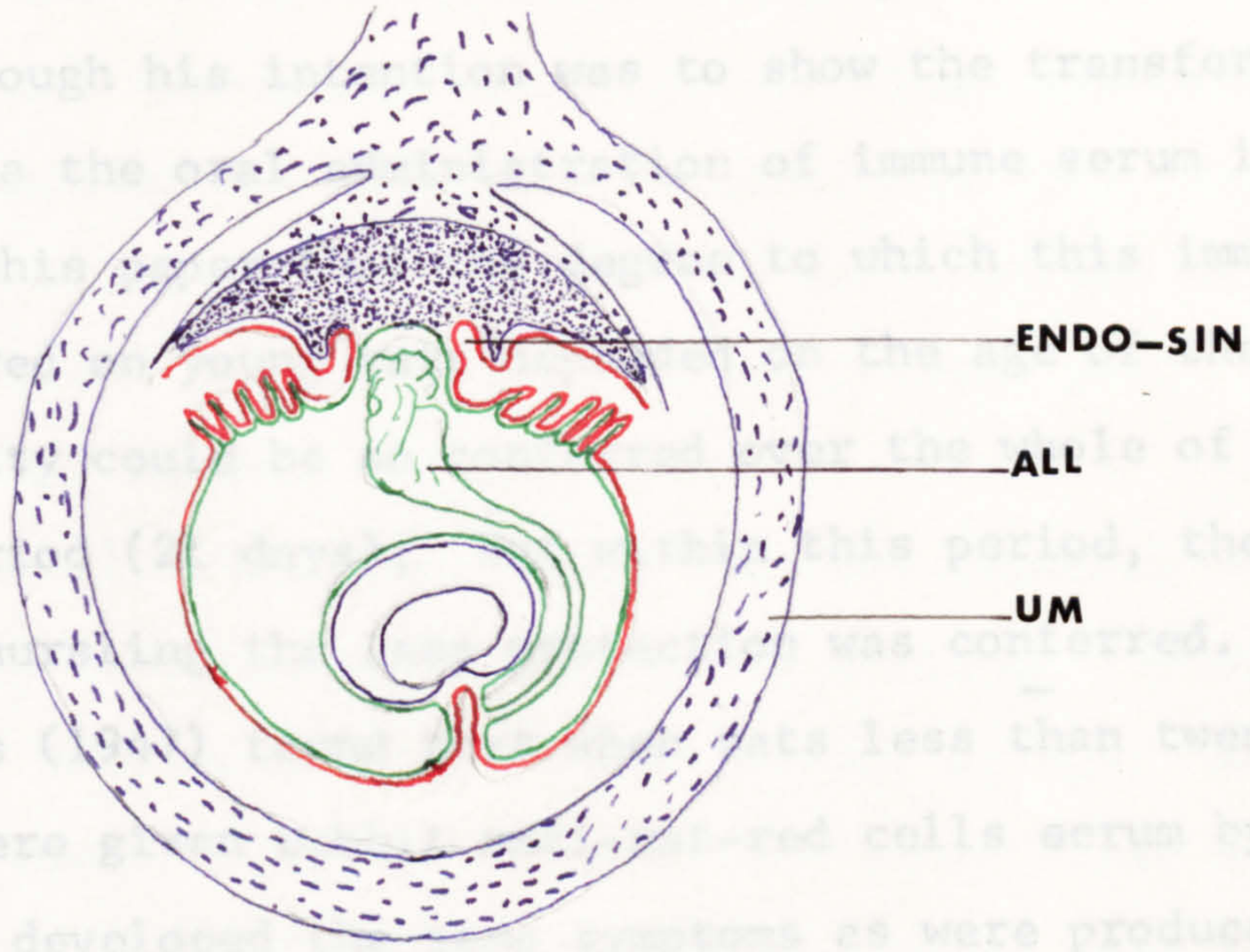
the chorionic mesoderm. (Figs 6d, 7a).

The endoderm, according to Duval (1892), and Brambell and Halliday (1956) gives off finger-like sinuses that enter into the depth of the placenta along the allantoic vessels (Fig. 7a).

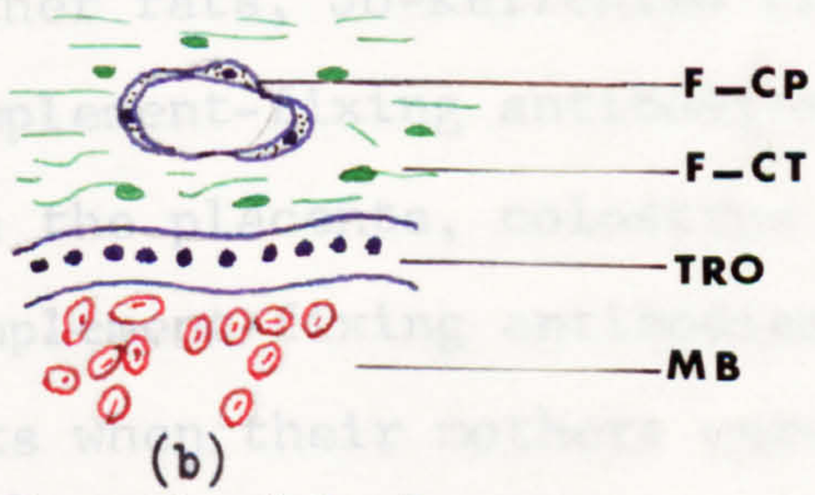
Mossman (1926, 1937) classified the placenta in the rat, mouse, guinea-pig and rabbit ad haemoendothelial, reviving the old observations of Duval (1889-1890) and Chipman (1903) quoted by Brambell et al. (1951) that suggested that only the foetal capillary endothelium intervenes between the foetal and maternal circulation in these animals. Recent work, especially electron-microscope studies of these difficult tissues has shown that in the rat, mouse and rabbit a layer of cellular trophoblast is present as well as foetal capillary endothelium, together with a small quantity of collagen (Wislocki, Deane and Dempsey, 1946; Amoroso, 1952; Wislocki and Dempsey, 1955).

Transfer of immunity in rats

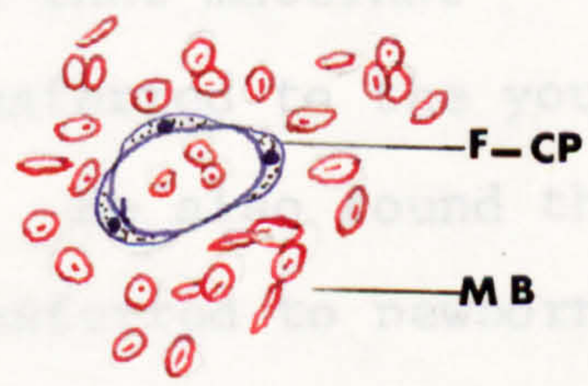
It was found by Culbertson (1938, 1939 a,b) that immunity against Trypanosoma lewisi was transferred from mother rats to offspring through the placenta and milk. He also found that the intestine of normal nursling rats could absorb antibodies present in the milk of immune mothers for at least the first fifteen days after birth. This immunity transferred from mother rats to the young lasted for only



(a)



(b)
Haemochorial placenta



(c)
Haemoendothelial placenta

Figure 7

Late development of embryonic membranes and placenta of rat and mouse

ENDO-SIN, endodermal sinus; F-CP, foetal capillary; F-CT, foetal connective tissue; MB, maternal blood; for ALL, TRO and UM see figure 1. (a is after Mossman (1937) with some modification; b and c are after Amoroso, 1952).

a short time. Culbertson also showed that immunity to T. lewisi could be transferred via the ingestion of immune serum. Though his intention was to show the transfer of immunity via the oral administration of immune serum it is clear from his paper that the degree to which this immunity was conferred on young rats depended on the age of the rats. Some immunity could be so conferred over the whole of the nursing period (21 days); but within this period, the older the nursling the less protection was conferred.

Bessis (1947) found that when rats less than twenty days old were given rabbit anti-rat-red cells serum by mouth they developed the same symptoms as were produced by injection of the antiserum.

By cross-nursing experiments with immune and normal mother rats, Jo-Keiichiro (1953) found that maternal complement-fixing antibodies were transferred to the young via the placenta, colostrum and milk. He also found that complement-fixing antibodies were transferred to newborn rats when their mothers were passively immunized with rabbit antisera during lactation.

Halliday (1955b) studied the transmission of passive immunity from actively immunized rats to their young and the relative importance of the prenatal and the postnatal transmission of passive immunity. He found that there was a rise in the concentration of the passively acquired antibodies in the sera of the young from the seventeenth

day of gestation to the second day of lactation. The antibody titre remained relatively constant after the second day and throughout lactation at values approximately equal to those of the mother. He concluded that there was a transmission of significant but small amount of passive immunity before birth, but the greater part occurred after birth.

Halliday (1955a, 1956) found that the capacity of the gut of young rats to absorb antibodies declined rapidly between the ninteenth to the twentieth day of age; no absorption could be detected after the twenty-first day of age. Halliday found that the taking of solid food did not appear to be responsible for bringing about the change in the permeability to antibodies of the gut of the young rat. He also investigated into the capacity of young rats to produce circulating antibodies, and found that suckling rats at ten to twenty-three days of age were capable of producing comparatively high titres of circulating antibodies eight or ten days after a single injection with Salmonella pullorum.

Terry (1956) found that the transfer to rats of immunity against Plasmodium berghei occurred mainly if not entirely through the milk. This passive immunity acquired by young rats disappeared seven weeks after weaning. He also found that suckling rats fed with immune serum can absorb antibodies just as they did when fed with immune

milk. Young rats fed with immune serum absorbed no malarial antibodies through the gut after the age of 22 days.

Bruce-Chwatt and Gibson (1956) also found that the transfer of passive immunity from mother rats to their offspring was mainly associated with sucking; a certain amount of immunity was, however, transferred through the placenta.

Halliday and Kekwick (1957) examined the serum proteins of rats at ages when antibodies can be absorbed, by electrophoresis and ultracentrifugation. They showed that between 18 and 24 days of age there was a decline in the amount of γ-globulin; this is the period over which antibody absorption from the gut falls off and finally ceases.

Halliday (1959) was able to induce a premature decline in the absorption of antibodies through the gut of young rats by the administration of large doses of deoxycorticosterone acetate or cortisone acetate.

Morgan (1964) studied the passage of γ-globulin, albumin and transferrin* from maternal plasma to the foetus

---

*Transferrin is a serum glycoprotein of a molecular weight 83,000 which binds and transports iron. It is represented by three beta globulin bands in starch-gel electrophoresis (Bearn and Parker, 1964).

in the rat. He found relatively high concentrations of these proteins in the sera of foetal rats at six and twenty hours after their mothers had received an intravenous injection of each labelled protein on the twentieth day of gestation. He concluded that the three proteins were relatively easily transmitted from mother rats near the end of pregnancy to their foetuses. Gamma-globulin was transferred to a greater degree than albumin and transferrin, which were transmitted to a similar degree.

Quinlivan (1964b) injected ^{131}I-labelled γ-globulin intravenously into rats during the last three days of gestation and subcutaneously into rats during the first nine days post-partum. He found that the labelled γ-globulin was transferred at a rapid rate from mother to foetus in utero. He also found a direct relationship between the amount of labelled γ-globulin in the infant and the duration of suckling. Quinlivan (1964c) and Quinlivan, Contopoulos and Masouredis (1964) reported that if ^{131}I-labelled γ-globulin was injected into rat foetuses of nineteen to twenty-one days gestation after the foetal membranes had been stripped, the labelled γ-globulin passed from the foetal into the maternal circulation; they considered that the route of transfer was transplacental.

The route by which passive immunity is transferred from mother to foetus in the rat was investigated by Brambell and Halliday (1956). Surgical experiments were

performed on the foetuses in situ. They found that when homologous immune serum was injected into the uterine lumen of nineteen to twenty days pregnant rats it entered both the exocoelomic and amniotic fluids of the foetuses through the yolk-sac splanchnopleur. When homologous immune serum was fed by stomach tube to the foetus it was absorbed by the foetal gut and appeared in the circulation. They also found that antibodies entered into the circulation of the inverted yolk-sac splanchnopleur from immune rat serum to which the yolk-sac was exposed. When the gut and the yolk-sac splanchnopleur routes were excluded, antibodies still reached the foetal circulation. They suggested a third alternative, the cavities in the entodermal sinuses of Duval and the allantoic circulation; they, however, did not exclude the possibility of the direct passage of antibodies from the maternal circulation across the placenta.

Anderson (1959) found that proteins intravenously injected into pregnant rats are stopped at the trophoblast but pass into the yolk-sac cavity before the parietal wall of the yolk-sac ruptures. He suggested that the maternal blood vessels pass between the cells of the trophoblast to come into close contact with Reichert's membrane so that the proteins may cross this membrane into the yolk-sac cavity without traversing the trophoblast, and so become available to the visceral wall of the yolk-sac. He also

showed that the visceral entodermal epithelium could take up proteins.

The route of transfer of proteins in suckling rats after birth was studied by Clark (1959). He found that proteins administered orally to suckling rats were absorbed by the columnar absorptive cells of the jejunum and ileum, but not by those of the duodenum; he suggested that the process by which the absorptive cells ingest the proteins was one of pinocytosis.

Quinlivan (1964a) studied the route of transfer of homologous γ-globulin labelled with ^{131}I from mother to foetus in the rat between the nineteenth and twenty-first day of gestation. He removed the foetuses from their sacs and resected the membranes to the placental edge and ligated the vitelline vessels and compared the transfer of labelled globulin from the maternal circulation to the foetuses with that in foetuses with intact membranes. In another set of experiments he tied the mouths of foetuses and the vitelline circulation and resected the foetal membranes. His results were similar in all these experiments. He found that the transfer of γ-globulin labelled with ^{131}I from the maternal circulation to the allantoic circulation occurred "within" the placenta.

The selectivity of the gut of young rats has been investigated by many authors, but, so far as I am aware, there is no information in the literature about the

selectivity of the foetal membranes involved in the prenatal transmission of antibodies.

Halliday (1955a) studied the selectivity of the gut of young rats by feeding them with heterologous immune sera prepared in mice, rabbits, cows and fowls. He showed that some of the heterologous sera e.g., rabbit sera, were absorbed less readily than the homologous sera, and that some e.g., cow and fowl sera were not absorbed at all. Mouse antibodies were absorbed by the gut of young rats as readily as rat antibodies.

Bangham and Terry (1957a) found by feeding ^{131}I-labelled homologous and heterologous (rabbit and monkey) serum proteins to young rats at various ages that homologous globulins were absorbed more readily than the heterologous. The survival of heterologous (monkey) serum proteins absorbed by the gut of young rats was found by Bangham and Terry (1957b) to be as long or even longer than that of the absorbed homologous serum proteins.

Halliday and Kekwick (1960) showed that absorption by young rats of antibodies against a particular antigen depended on whether the antibodies were produced early or late in the immunization course. There was also a difference in absorption of antibodies against different antigens. Halliday and Kekwick felt that these differences depended on the fraction of the globulins in which the antibodies were located.

Transfer of immunity in mice:

Transmission of passive immunity to young mice is mainly through the milk; the amount transmitted by placental route is relatively small. This was found by Culbertson (1940) who showed that normal mice became immune to Trypanosoma duttoni if allowed to suck mothers that have recovered from an artificial infection with this organism. Culbertson also found that suckling mice can absorb antibodies present in the milk through the intestine until fifteen days of age.

Gordon and Curley (1949) reported the transmission of specific antibodies to mouse encephalomyelitis virus from actively immunized mice to adopted normal sucklings through the milk. The passive immunity acquired by the sucklings from the immune females lasted till they were three months old.

Thompson and Meyers (1950) found that passive immunity in mice was largely transferred by the milk; the possibility of transplacental immunity was not eliminated. Dean (1952) found similar results in the transfer of passive immunity against mouse encephalomyelitis.

Morris (1958a) produced haemolytic anaemia in young mice by feeding them with rabbit anti-mouse-red-cell serum.

Kaliss, Dagg and Stimpfling (1963) found that haemagglutinating and "enhancing" antibodies were transferred from mother mouse to foetus and suckling. The

gut of young mouse was capable of absorbing antibodies up to at least fifteen days of age.

Kosumen and Halonen (1963) found that in young mice passive immunity against tetanus was transferred both *in utero* and by milk, the latter being the main route.

The route of transmission of passive immunity to the foetus in mice probably resembles closely that of the rat (Brambell, 1958).

The selectivity of the foetal membranes in antibody transfer to the circulation is probably similar to that of the rat.

SUMMARY

1. The young of rats and mice acquire their passive immunity pre- and postnatally; the latter being the main route.

2. Suckling rats and mice are capable of absorbing antibodies present in milk and immune sera.

3. The ability of the suckling to absorb antibodies through the gut is lost after twenty and fifteen days of age in rats and mice respectively.

4. The loss of antibody absorbing capacity by the young could be induced prematurely by the administration of cortical steroids.

5. The passive immunity acquired by young rats disappears about seven weeks after weaning.

6. Suckling rats at ten to twenty-three days of age are

capable of producing antibodies after immunization with antigens.

7. The route of transfer of passive immunity from mother to foetus in rats and probably in mice could be either the foetal gut, the yolk-sac splanchnopleur or the placenta.

8. The route of transfer of passive immunity in suckling rats and mice is via the columnar cells of the jejunum and ileum and probably into the peripheral circulation via the lymphatic circulation.

9. The gut of young rats, unlike that of calves, pigs and lambs is selective in the absorption of antibodies.

PRE-NATAL TRANSFER OF IMMUNITY

Transfer of immunity from mother to young in rabbit, guinea-pig and man is mainly prenatal; in these mammals colostrum plays a negligible role in the transfer of immunity from mother to young. At birth the concentration of passively acquired antibodies may be equal to, or even greater than, that in the mother (Ratner, Jackson and Gruehl, 1927; Schneider and Szathmary, 1939c, 1940).

MAN

Foetal membranes and placentation, (largely based on Hamilton, Boyd and Mossman; Human embryology, 1962):

Owing to accumulation of fluid in the intracellular spaces of the inner cell mass and the appearance of a large cavity, the blastocoel (fig. 8a), the morula gradually changes into a blastocyst. As a result of the appearance of the blastocoele the inner cell mass is pushed eccentrically and becomes attached to the inner side of the trophoblast.

The endoderm differentiates from the blastocoelic surface of the inner cell mass and does not completely line the trophoblasts; thus the blastocyst of man is unilaminar. The rest of the inner cell mass arranges itself to form a disc, the embryonic disc. A primary yolk-sac begins to form with the endodermal cells forming its roof and the rest is formed by mesothelial-like endodermal cells (Fig. 8b). The secondary yolk-sac is formed when

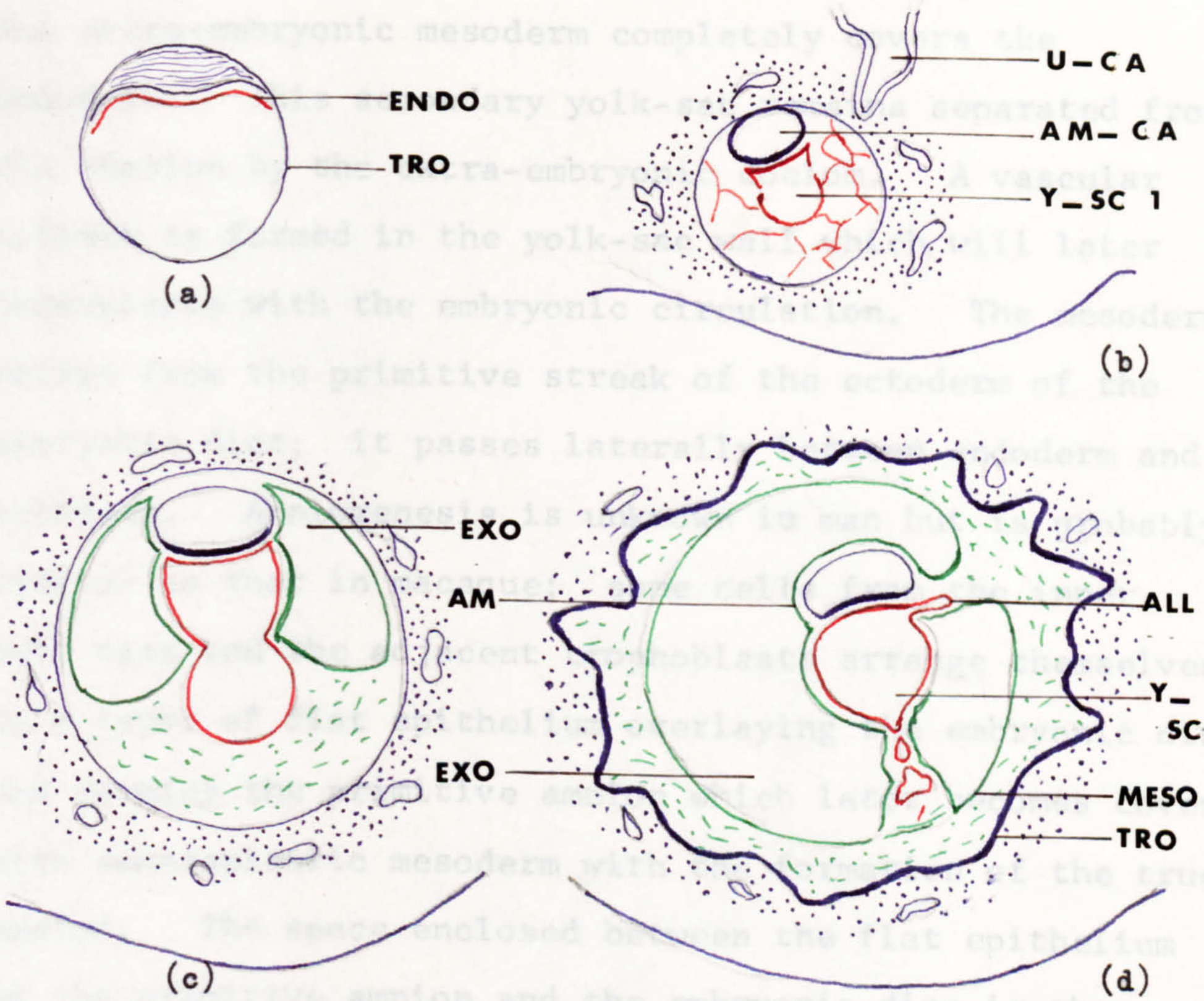


Figure 8

Blastocyst and early development of embryonic membranes of man

AM, amnion; AM-CA, amniotic cavity; U-CA, uterine cavity; Y-SC 1 and 2, primary and secondary yolk-sac; for ENDO, EXO, MESO and TRO, see figure 1. (a, b, c and d are after Hamilton, Boyd and Mossman, 1962).

the extra-embryonic mesoderm completely covers the endoderm. This secondary yolk-sac remains separated from the chorion by the extra-embryonic coelom. A vascular network is formed in the yolk-sac wall which will later communicate with the embryonic circulation. The mesoderm arises from the primitive streak of the ectoderm of the embryonic disc; it passes laterally between endoderm and ectoderm. Amniogenesis is unknown in man but is probably similar to that in macaque; some cells from the inner cell mass and the adjacent trophoblasts arrange themselves in a layer of flat epithelium overlaying the embryonic disc and forming the primitive amnion which later becomes covered with somatopleuric mesoderm with the formation of the true amnion. The space enclosed between the flat epithelium of the primitive amnion and the embryonic disc is the amniotic cavity which later becomes filled with fluid. The chorion is formed when the trophoblasts become lined with mesoderm. The allantois arises as a diverticulum from the caudal wall of the yolk-sac; it does not reach to the chorion nor does it enlarge to form an allantoic vesicle (Fig. 8d, 9a and b).

Implantation takes place at seven to eight days after ovulation; it is of the interstitial type in which the blastocyst becomes completely embedded in the uterine mucosa and a decidua is formed which persists till term. Thus the only embryonic surface that is exposed to the

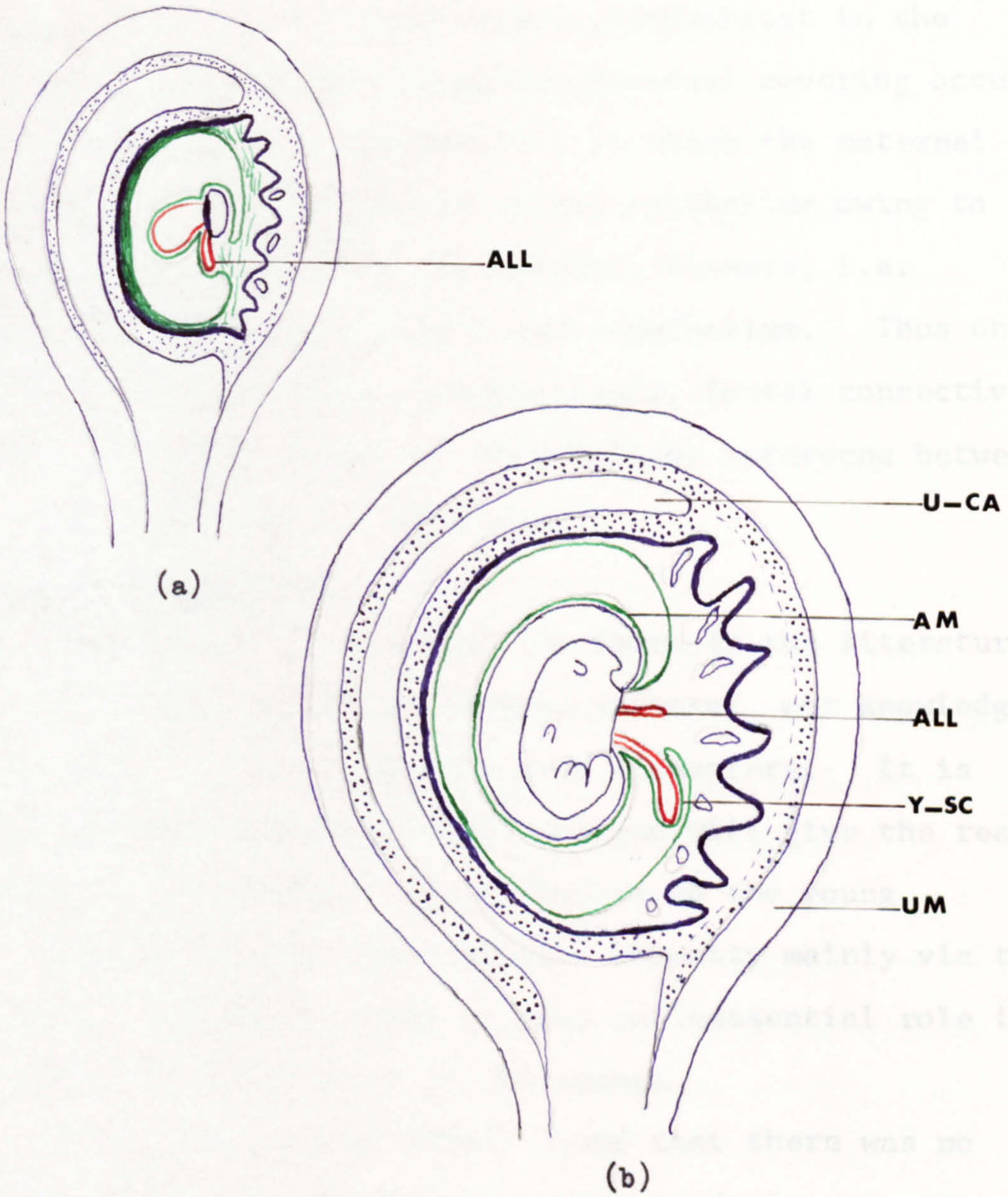


Figure 9

Embryonic membranes and placentation of man

ALL, allantois; AM, amnion; U-CA, uterine cavity; UM, uterine muscle; Y-SC, yolk-sac (after Hamilton, Boyd and Mossman, 1962).

uterine cavity is the blastocystic trophoblast in the very short period before complete decidual covering occurs.

The placenta is haemochorial in which the maternal blood directly bathes the chorionic epithelium owing to the disappearance of all the maternal tissues, i.e. epithelium, connective tissue and endothelium. Thus only the three foetal tissues (trophoblasts, foetal connective tissue and foetal capillary endothelium) intervene between maternal and foetal circulations.

Transfer of immunity:

A great deal of research was found in the literature about the immune state of newborn infants; our knowledge is, however, in many respects very incomplete. It is hoped that the following short account will give the reader an idea of the transfer of antibodies to the young.

Infants acquire their passive immunity mainly via the placenta; colostrum seems to play an inessential role in the transfer of immunity to the young.

Kuttner and Ratner (1923) found that there was no measurable loss of diphtheria antitoxin during passage through the placenta in that the cord blood antitoxin corresponded to that of the mother. They also found that the concentration of antitoxin occasionally present in human colostrum was always less than that of the mother's serum or cord blood; no increase in antitoxin concentration of the infant's blood was found after colostral feeding.

They concluded that antibody transfer from mother to infant was placental; colostral feeding was inessential in human infants.

Vahlquist and Högstedt (1949) by feeding human diphtheria antitoxin to newborn infants starting from twelve to twenty-four hours of age found that minute amounts of antitoxin were absorbed during the first week of life. They concluded that breast milk was of little value for the transfer of diphtheria antitoxin from mother to young.

Nordbring (1957) by feeding premature infants soon after birth with heterologous (cow and sow) colostrum, containing Salm. paratyphi H agglutinins, found that poor absorption of antibodies occurred. He concluded that colostrum played a negligible role in the transfer of immunity to infants.

Vahlquist (1960) stated that in man transfer of antibody and γ-globulin from mother to young was via the placenta; colostrum played an unimportant role in the transfer.

Newborn infants possess considerable amounts of euglobulin at birth (Pedersen, 1945). By electrophoretic examination of ten pairs of maternal and foetal plasmas taken at birth, Longsworth, Curtis and Pembroke (1945) found that both the absolute and the relative γ-globulin concentration in the foetus was higher than that in either their normal mothers or in normal non-pregnant adults.

Remington and Pickford (1947) found that there was an increase in globulin in premature infants from twenty weeks to full term.

Moore, Du Pan and Buxton (1949) found that newborn infants possessed high levels of γ-globulin at birth; this level decreased markedly during the first two months of postnatal life.

The immunity of the newborn depends a great deal on that of the mother (Vahlquist, 1960). Antibody transfer across the placenta is not always protective to the foetus against certain diseases; it certainly results in the destruction of the foetus in certain cases of the haemolytic disease of the newborn. The discovery of the role of the blood-group substances in haemolytic disease (Levine, Newark and Stetson, 1939; Landsteiner and Weiner, 1940) lead to the understanding of many clinical problems of this disorder. Haemolytic disease results from the immunization of the mother by antigens present on the foetal red blood cells, inherited from the father and absent in the mother, and the transfer of the maternal antibodies to the foetus.

Brambell, Brierly, Halliday and Hemmings (1954) suggested that the route of transfer of antibodies from maternal to foetal circulation was the amniotic fluid and foetal gut. It was, however, found by Wasz-Hockert, Wager, Hautala and Widholm (1956) that the level of diphtheria antitoxin in the sera of newborn infants with

oesophageal atresia was comparable to that of the mother. They concluded that the swallowing of amniotic fluid by the foetus was not the route of antibody transfer from mother to foetus. However, this finding did not preclude the possibility of antibody transfer from the amniotic fluid via the naso-pharynx and respiratory tract (Brambell, 1958).

Dancis, Lind, Oratz, Smolens and Vara (1961) injected γ-globulin labelled with radioactive iodine into the amniotic sacs of two 3-months pregnant women; they found that twenty-four hours later radioactivity was significant in the foetal blood but much lower than that present in the amniotic fluid. In another experiment Dancis et al. injected human tetanus antiserum in the amniotic fluid and found that no antitetanus antibodies were detectable in the cord serum. They thus excluded the possibility of transfer of proteins from mother to foetus through the amniotic fluid and gastroentestinal tract, and concluded that the placenta was the only other route for transfer of proteins.

Transfer of antibodies from maternal to foetal circulation is evidently selective in nature; certain antibodies pass freely into the foetal circulation whilst others pass less freely or not at all. The hypothesis that the human placenta was selective in nature was put forward by Hartley (1948) to explain an observation on a case reported by Chesney (1945); refined horse diphtheria

antiserum injected into a pregnant woman was not transferred to her foetus. Hartley was in fact the first to show that antibody transfer from mother to foetus is selective in character.

Hartley (1951) explained the selectivity of transfer of antibodies from mother to foetus on the basis or origin of these antibodies; homologous antibodies were transferred more readily than heterologous.

The human placenta is selective not only for homologous antibodies but also for types of antibodies.

Timmerman (1931) found that typhoid H agglutinins were readily transmitted from mother to foetus while O agglutinins were not.

Wiener (1948) observed that incomplete (blocking) Rh antibodies readily passed through the placenta whilst complete Rh antibodies did not.

Zuelzer and Kaplan (1954) found that both complete and incomplete anti-A and anti-B isoantibodies passed through the placenta; the incomplete isoantibodies passed more readily.

Franklin and Kunkel (1958) compared the levels of high molecular weight (19S) γ-globulins in maternal and umbilical cord. They found that the serum of the newborn contained much lower concentration of high molecular weight fraction of γ-globulin (19S) than that of the adult.

Gelford, Strean, Pavilanis and Sternberg (1960)

examined the permeability of the placenta to various constituents of different molecular weights and sizes like antibodies, proteins, lipoproteins and cholesterol. They showed that there was no relationship between the rate of transfer of the various constituents and the molecular size or weight. The authors, however, pointed out that only in the case of antibodies could they decide whether they were of foetal or maternal origin; metabilites (protein, lipoprotein and cholesterol) might be of maternal or foetal origin.

Vahlquist (1960) divided antibodies into three groups according to their passage through the placenta. To the first group in which antibodies passed readily leading to equal maternal and foetal titres belonged the antitoxins (diphtheria and tetanus), antihaemolysins (antistreptolysin, antistaphylolysin), antiviral antibodies (measles, poliomyelitis) some complement-fixing antibodies (influenza, toxoplasmosis) and incomplete Rh agglutinins. To the second group in which antibodies passed less readily leading to lower foetal titres than maternal belonged antibacterial antibodies (H. influenza and dysentery), some complement-fixing antibodies (syphilis) and iso-agglutinins (anti-A and anti-B). To the third group in which antibodies did not cross the placenta belonged some antibacterial antibodies and complete Rh agglutinins.

Freda (1962) suggested that the selectivity of the

placenta in antibody transfer can be best explained in terms of heterogeniety of antibodies (a single antigen may provoke the formation of a heterogeneous population of antibodies that differ in physical, chemical and/or immunological properties). Not all antibodies in a single population are able to cross the placenta.

Gitlin, Kumate, Urrusti and Morales (1964) investigated the selectivity of the placenta in the transfer of several labelled human proteins of different molecular weights and fragments of papain-hydrolysed 7S γ_2-globulin in pregnant women. The proteins studied were: acid glycoproteins, albumin, transferrin, 7S γ_2-gloublins, fibrinogen, 19S macroglobulin, the F fragment of the 7S γ_2-globulin (Porter's fragment III), the S fragment of the 7S γ_2-globulin (Porter's fragments I and II) and 1S to 3S urine γ-globulin (γ_u-globulin). The specific ^{131}I-labelled protein was injected intravenously into the pregnant woman from four weeks to a few minutes before delivery. The protein-bound radioactivity was determined in the mothers plasma before and during delivery, in the infant's plasma and in the amniotic fluid when available. Gitlin et al. found that all the plasma proteins studied were transferred to the foetus but in different amounts. Little or no labelled 19S macroglobulin, transferrin, fibrinogen and albumin was found in the infant's plasma in some cases. The concentration of the F fragment in

the foetal sera relative to those of the mother was higher than that of the S fragment. According to the authors the transfer of plasma proteins from mother to young in man appeared to be via the placenta.

Formation of antibodies by the young was studied by Osborn, Dancis and Julia (1952a,b). These authors immunized infants from one week to six months of age, who had no passively acquired antitoxin, with one injection of diphtheria alum-precipitated toxoid as well as of tetanus. They found that infants can form antibodies from birth and their capacity for antibody formation improves during early life. Osborn et al. also studied antibody formation by infants who had measurable titres of transplacentally acquired diphtheria antitoxins. Infants from two weeks to six months of age were immunized with one injection of alum-precipitated diphtheria toxoid. They found that antibody formation by infants who had high level of passively acquired antitoxin was depressed while those with low level of passively acquired antitoxin responded to immunization as those who had no passively acquired antitoxin. These authors suggested that interference with active immunization depended on the titre of the passively acquired antibodies and the potency of the antigen used.

Barr, Glenny and Randall (1949) studied the rate of loss of passively acquired diphtheria antitoxin by newborn

children. They found that the antibody titre fell at a logarithmic rate.

Barr, Glenny and Randall (1950) studied the effect of the amount of passively acquired diphtheria antitoxin on the response to immunization by two doses, each of 0.5 ml of alum-precipitated toxoid (50 Lf per ml.) in newborn human babies. They found that when the level of antitoxin acquired from the mother had fallen below 0.04 unit per ml. at the time of the first injection, no interference with the active immunization was observed; when the level was above 0.1 these antibodies interfered with active immunization. Barr et al. also studied the effect of age and interval between doses on the active immunization of newborn babies. They suggested that a poorer response was produced by babies whose immunization was started after six months of age. In babies of less than six months of age a better response was obtained when the interval between the first and second dose was 10 - 18 weeks than when the interval was 6 - 9 weeks. They suggested that babies could be successfully immunized with three injections given at three, six and eighteen months of age.

SUMMARY

1. Transfer of immunity from mother to foetus in man occurs before and not after birth.

2. At birth newborn infants possess high levels of

γ-globulin.

3. Antibody transfer across the placenta may lead to many haemolytic disorders.

4. The route of antibody transfer in man is most probably via the placenta and not via the amniotic fluid and foetal gut.

5. The placenta of man is highly selective in nature. It differentiates not only between homologous and heterologous antibodies but also between types of antibodies.

6. Antibody formation by infants is possible from birth.

7. Transplacentally-acquired antibodies by infants may interfere with the formation of antibodies as a result of active immunization.

THE RABBIT

Foetal membranes and placentation (mainly based on a more detailed description by Brambell, Hemmings and Henderson, 1951; Antibodies and embryos, Athlone press).

The blastocyst of the rabbit, just before implantation, is a spherical vesicle with two layers, the trophoblast with a thickened embryonic shield and the endoderm lining the trophoblast from the region of the embryonic shield to about one-third of the way downwards (Fig 10a).

Implantation is "central" and occurs on the seventh day of gestation. The blastocyst attaches itself by localized thickenings of the trophoblasts to the uterine mucosa at several points over the antimesoetrial area. Fusion areas are formed at the regions of attachment of the trophoblasts with the degenerated uterine epithelium. At this time the endoderm has completely lined the trophoblast forming the bilaminar omphalopleur. The mesoderm in the meantime grows from the primitive streak to as far as the equator of the blastocyst between the two layers of the omphalopleur leaving the other hemisphere of the blastocyst undisturbed. A cavity develops in the mesoderm, the exocoelic cavity, with the nonvascular chorion forming its roof and the vascular yolk-sac splanchnopleur forming its floor (Fig. 10b). The blastocyst increase in size by expansion over the yolk-sac cavity. The amnion is

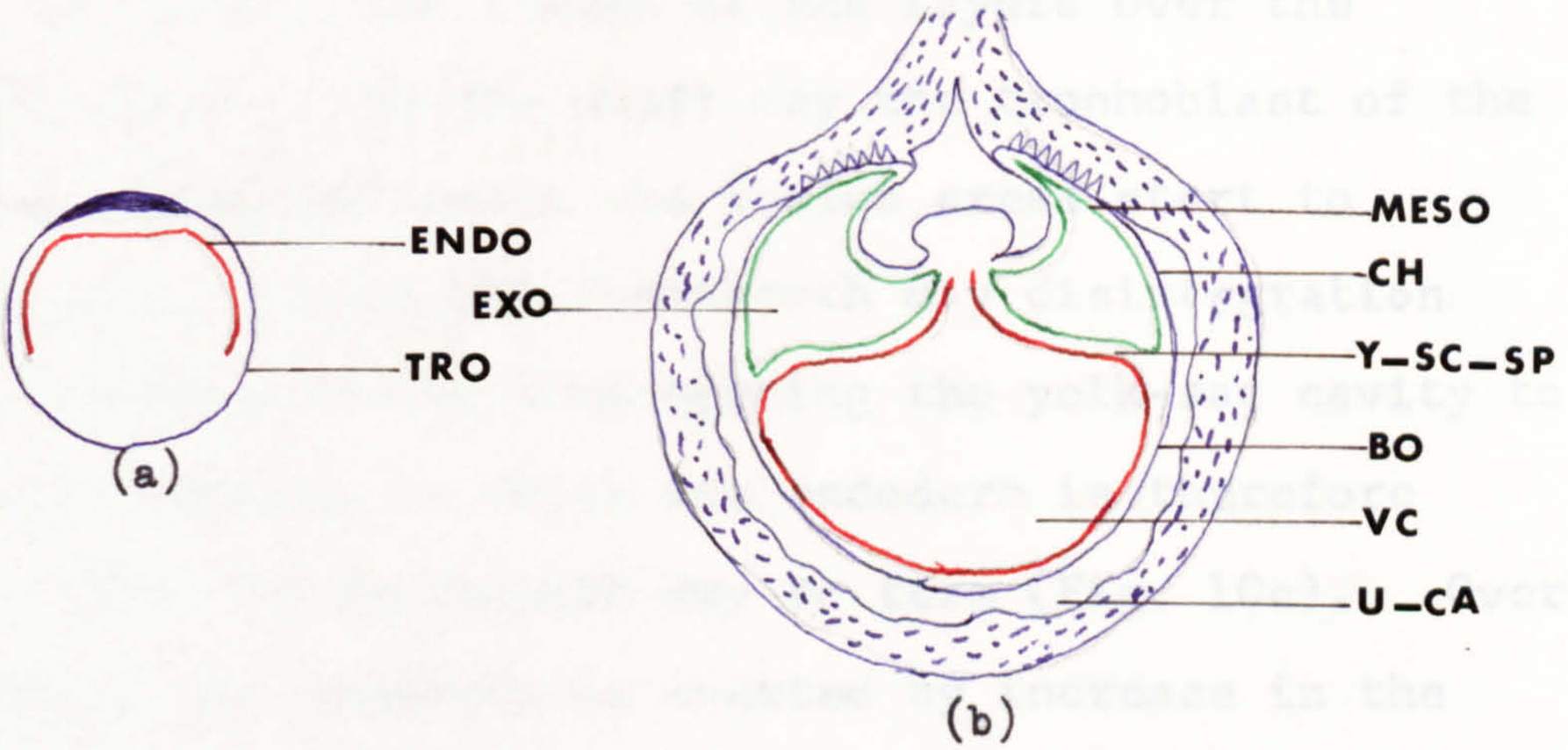


Figure 10

Blastocyst and development of embryonic membranes of rabbit

BO-R, bilaminar omphalopleur remnant; CH, chorion; CH-L, "chorion laeve"; ST, sinus terminalis; Y-SC-SP, yolk-sac splanchnopleur; U-CA, uterine cavity; for ALL, BO, ENDO, EXO, MESO, TRO, UM and VC see figure 1. (a and b are after Brambell, 1948; c is after Morris, 1950).

formed by folding and fusion of the layers over the embryonic disc. By the ninth day the trophoblast of the bilaminar omphalopleur at the fusion areas start to disintegrate, and on the fourteenth day disintegration of the endoderm occurs thus opening the yolk-sac cavity to the uterine lumen, to which its endoderm is therefore exposed from the fourteenth day to term (Fig. 10c). Over this period the endoderm is everted by increase in the exocoel fluid. By this exposure of the endoderm the rabbit, like the guinea-pig, rat and mouse exhibits an inversion of the germ layers. The remnants of the bilaminar omphalopleur can be seen at both sides of the sinus terminalis. The allantois rapidly grows into the exocoele until it comes in contact with the central part of the chorion leaving small marginal zones on both sides "chorion laeve". (Amoroso, 1952, Marshall's physiology of reproduction).

The surfaces exposed to the uterine lumen are thus, in turn, the bilaminar omphalopleur, the chorion and the bilaminar omphalopleur, and after the fourteenth day, the chorion and endoderm of the yolk-sac splanchnopleur.

Mossman (1926) classified the placenta in the rabbit as haemoendothelial with only the foetal capillary endothelium intervening between the foetal and maternal circulation. Recent work, however, has shown that the placenta in the rabbit is haemochorial; all the foetal layers are present. These are the trophoblast, foetal

connective tissue and foetal capillary endothelium (for details and references see foetal membranes and placentation in rat and mouse).

Transfer of immunity:

Transfer of passive immunity from mother to young in rabbits takes place mainly or entirely before birth.

Rodolfo (1934) determined the transfer of agglutinins and haemolysins from mother to young in rabbits between twenty-two and thirty days of pregnancy. He concluded that the permeability of the placenta changed as pregnancy advanced; this change was due to the change in its histological structure.

Schneider and Szathmary (1939c) immunized rabbits against typhoid and diphtheria and found that the antibacterial and antitoxic antibodies were transmitted to the foetus via the placenta. Sucking did not enhance the immunity of the newborn.

Prenatal transfer of cholera antibodies from mother rabbits immunized with live vibrio vaccine to her young was demonstrated by Fanse and Dutta (1964).

Studies of foetal sera and the transfer of serum fractions from mother to foetus were made by many authors mainly by Brambell and colleagues.

Pedersen (1945) found by ammonium sulphate fractionation that rabbit foetal sera contained little fetuin but much normal globulin.

Brambell and Mills (1947) during their studies on

sterility and prenatal mortality in wild rabbits found fibrinogen in the yolk-sac fluid of seven- to twelve-day embryos. Further electrophoretic, ultracentrifugal and immunological studies in Brambell's laboratory showed that α-, β- and γ-globulin and fibrinogen were present in yolk-sac fluid in proportions similar to those in maternal. plasma. It was also found that actively or passively acquired homologous and heterologous agglutinins in the maternal circulation pass freely into the yolk-sac cavities of seven- and eight-day embryos before the formation of the allanto-chorionic placenta. Brambell and colleagues implicated the bilaminar omphalopleur as the membrane through which these agglutinins pass into the yolk-sac cavity. They concluded that the bilaminar omphalopleur is non-selective in nature since it did not discriminate between homologous and heterologous proteins (Brambell, Hemmings and Rowland 1947, 1948; Brambell and Hemmings, 1949, with an addendum by McCarthy and Kekwick).

Brambell, Hemmings, Henderson and Kekwick (1953) found by electrophoretic and ultracentrifugal examinations of foetal sera that components corresponding to albumin, α-, β- and γ-globulins were present, but the proportion of these components and the total protein concentration was different from that of the adult rabbit.

Winkler, Fitzpatrick and Finnerty (1958) found that homologous albumin was transferred from pregnant rabbits

to their foetuses at term at a very slow rate during the first twenty-four hours after its injection into the maternal circulation.

Kulangara and Schjeide (1962) by intravenous injections of whole rabbit serum containing labelled cystine into twenty-four-day pregnant rabbits found that increasing amounts of protein passed into the foetal circulation with the passage of time.

Morgan (1964) studied the transfer of transferrin*, albumin and γ-globulin from the maternal plasma to the foetus in rabbits on the twenty-eighth day of pregnancy. He found relatively high concentrations of γ-globulin and albumin in the foetal sera; the globulin value was approximately twice that of albumin. The transferrin concentration in the foetal sera was only about one-twentieth that of albumin. He concluded that in rabbits near term the transmission of γ-globulin and albumin from mother to foetus occurred more readily than that of transferrin.

The route of transmission of maternal antibodies and serum proteins was studied by Brambell, Hemmings, Henderson, Parry and Rowlands (1949) in a series of experiments. They found that the allantochorionic placenta played no role in the transfer of antibodies from nother to young in

---

*For properties see rat and mouse section, page 53.

the rabbit, and that the yolk-sac splanchnopleur played a primary role in the transfer of antibodies by direct absorption from the uterine lumen. They first showed that antibodies normally passed into the uterine lumen without the need for the presence of placenta or embryos, and that antibodies injected into the uterine cavity were absorbed by the foetus. However, if the vitelline circulation was interrupted, even though the placental circulation was intact, no antibody was absorbed by the foetus from the uterine lumen. They concluded that antibodies passed from the maternal circulation into the uterine lumen then into the foetal circulation via the yolk-sac splanchnopleur and the vitelline circulation. The discovery of Brambell et al. of the transfer of antibodies from mother to foetus in the rabbit by this route led to questioning of the importance of the placenta in the transfer of passive immunity to the young, and started many fields of new research.

Kulangara and Schechtman (1962) found that when human serum albumin was injected intravenously into pregnant rabbits between nineteen and twenty-eight days post coitum after ligation of the vitelline vessels of some of the foetuses, the injected proteins appeared in the circulation only of those foetuses whose vitelline vessels had not been ligated.

The selective entry of antibodies and serum proteins

into the foetal circulation of the rabbit has been investigated by many authors particularly by Brambell and his colleagues who investigated the mechanism of this selection.

It was found by Holford (1930) that egg albumin, horse or bovine serum or globulins were transmitted to the foetal circulation after injection into pregnant rabbits near full term, whereas horse haemoglobin was excluded.

Brambell, Hemmings, Henderson and Rowland (1950) by intra-uterine injections of rabbit and bovine anti-Brucella agglutinins and of equine diphtheria antitoxin at twenty-four days post-coitum showed that the yolk-sac splanchnopleur of the rabbit embryo freely admits to the foetal circulation the homologous antibodies whereas the heterologous antibodies were almost, but not entirely, excluded. They also found that this selectivity (like that of the human placenta found by Hartley, 1948) of the yolk-sac splanchnopleur did not depend on the molecular size of the injected antibodies but on the species of origin. In embryos of twenty days post-coitum the selectivity of the yolk-sac splanchnopleur between rabbit and bovine agglutinins was found by Brambell *et al.* to be relatively slight.

Cohen (1950) studied the transfer of bovine and human serum γ-globulin and rabbit antibovine and antihuman γ-globulin to the foetus after intravenous injections into a series of pregnant rabbits near term. By

precipitation tests he found that transmission of heterologous serum γ-globulin from mother to young differed from that of homologous antibodies. There was a delay in transfer of human and bovine γ-globulin and the equilibrium between maternal and foetal titres was not reached until five days after the administration of these globulins in the maternal circulation. However, there was a rapid transfer of homologous antiserum; the equilibrium between maternal and foetal sera was reached at four hours after administration.

Brambell, Hemmings, Henderson and Oakley (1952) measured the relative rate of entry of antitoxins prepared in rabbits, cattle and horses into the foetal circulation and into the amniotic fluid from the uterine lumen of rabbits on the twenty-fourth day of gestation. They found that homologous antitoxin entered the foetal circulation at a rate at least fifty times greater than the heterologous, however both homologous and heterologous antitoxins entered the amniotic fluid at almost identical rates.

Batty, Brambell, Hemmings and Oakley (1954) investigated further the selective admission of antitoxins, injected into the uterine cavity, into the foetal circulation of rabbits. Antitoxins prepared in rabbit, man, guinea-pig, dog, horse and cow were used and their concentrations in the foetal sera were corrected to unit

concentration in the serum administered. They found that the species in which an antitoxin is produced affected the entry and concentration of the antitoxin in the foetal circulation. Antitoxin produced in rabbit, man, guinea-pig, dog, horse and cow were present in the foetal serum at decreasing concentrations in that order. However, the entry of antitoxins into the amniotic fluid, stomach contents and maternal serum (from the uterine lumen) was not affected by species of origin of these antitoxins.

Brambell, Hemmings and Oakley (1954) compared the transmission of natural and pepsin-digested rabbit antitoxin from the uterine cavity into the foetal circulation of twenty-four-day pregnant rabbits. A mixture of these two antitoxins was prepared in each experiment so that the foetuses were exposed simultaneously to the natural and pepsin-digested antibodies. They found that natural tetanus antitoxin was transmitted to the foetus more readily than the pepsin-digested antitoxin. The reduction in size by pepsin digestion, therefore, did not increase the rate of transfer.

Brambell, Hemmings, Oakley and Porter (1960) by papain digestion of immune rabbit γ-globulin isolated three fractions. Fractions I and II retained the antibody activity, whereas fraction III retained the antigenic specificity of the original molecule. Brambell et al. (1960) studied the relative transmission of these

three fractions from the uterine cavity of twenty-four-day pregnant rabbits to the foetal circulation. They found that "fraction III was transmitted nearly as readily as whole γ-globulin, whereas fractions II and I were transmitted only 1/5 to 1/10 as readily, respectively, as fraction III". They suggested that the fraction which contained the antigenic group of the original molecule, i.e. fraction III, possessed the configuration recognized by the cells as homologous γ-globulin.

Hemmings and Jones (1963) by ultracentrifugation in a gradient of sucrose confirmed the finding that molecular size does not play an important part in transmission of immunity from mother to foetus in rabbits. Brucella abortus agglutinins that were readily transmitted to the foetus were in the light component; they were not detected in the macroglobulin. On the other hand rabbit anti-human-red-cell agglutinins, which were confined to the macroglobulin component of the serum, were also transferred to the foetal circulation.

Morgan (1964) during his studies of the passage of maternal plasma proteins to the foetus in rabbits also concluded that transmission did not depend on molecular size. He found that γ-globulin was transmitted to the foetus more readily than albumin and transferrin.

The mechanism of selection was investigated by Brambell and colleagues. For the antibodies to reach the

foetal circulation from the uterine lumen via the yolk-sac splanchnopleur they must traverse the following layers: the yolk-sac endoderm, the splanchnic mesenchyme and the vascular endothelium (Brambell, Hemmings and Henderson, 1951). It was found that the heterologous antibodies were neither retained in the foetal tissue nor were degraded by them; heterologous globulins were not excluded at the cell surface of the yolk-sac endoderm nor were they treated in different manner from homologous globulins once they entered the foetal circulation. It was suggested that both homologous and heterologous globulins were taken up equally by the foetal cells and selection occurred when these proteins left the cells to enter the foetal circulation. A small part of the proteins present in the foetal cells passed into the circulation and the rest was broken down. The quantity of the protein that passed into the foetal circulation varied with the species of origin (Brambell, Hemmings, Henderson and Oakley, 1952; Hemmings, 1956; Hemmings and Oakley, 1957; Hemmings, 1958).

SUMMARY

1. Transfer of passive immunity from mother to young in rabbit occurs mainly before birth.

2. The route of transfer of antibodies from mother to foetus is as follows: maternal circulation, uterine cavity, yolk-sac splanchnopleur, vitelline circulation and foetal

circulation; it is not via the allanto-chorionic placenta.

3. Maternal plasma proteins and homologous and heterologous agglutinins pass into the yolk-sac cavities of seven and eight day embryos before the formation of the allanto-chorionic placenta; this passage is non selective.

4. Rabbit foetuses possess albumin, α-, β-, and γ-globulin in their sera.

5. Endoderm of the yolk-sac splanchnopleur is selective in nature allowing homologous proteins to pass more readily than heterologous.

6. Selection does not depend on molecular size but on species of origin of the molecule.

7. Entry of proteins into the amniotic fluid is non-selective.

8. Heterologous proteins once in the foetal circulation are treated like homologous during the first twenty-four hours.

9. Selection occurs when the proteins leave the foetal cells to enter the circulation.

GUINEA-PIG

Foetal membranes and placentation (mainly after Sansom and Hill, 1931 and Duval, 1892):

The guinea-pig blastocyst consists of a layer of trophoblast covering a cavity, the blastocoel, and at one pole only, the inner cell mass. As the blastocyst enlarges, endoderm differentiates from the inner part of the inner cell mass; it never extends along the parietal trophoblast (Fig. 11a). The trophoblast covering the inner cell mass thickens to form the ectoplacental mass or "Träger", within which develops the ectoplacental cavity. A split now develops in the inner cell mass, and the accumulation of fluid in it pushes the deeper part of the inner cell mass (the embryonic mass) away from the "Träger", carrying the endoderm along with it (Fig 11b). The endoderm grows pari passu with the expansion of this proexocoelic cavity. The parietal trophoblast, which has never been covered with endoderm degenerates and disappears by the eighth day, so that the endoderm covering the pro-exocoel is now exposed to the uterine mucosa. By the fourteenth day the cavity of the pro-exocoel becomes lined with mesoderm. The amnion develops as a split in the embryonic mass, and as the embryo develops it is forced upwards into the exocoel, and the endoderm is pinched up to form the embryonic gut (Fig. 12a). The final result

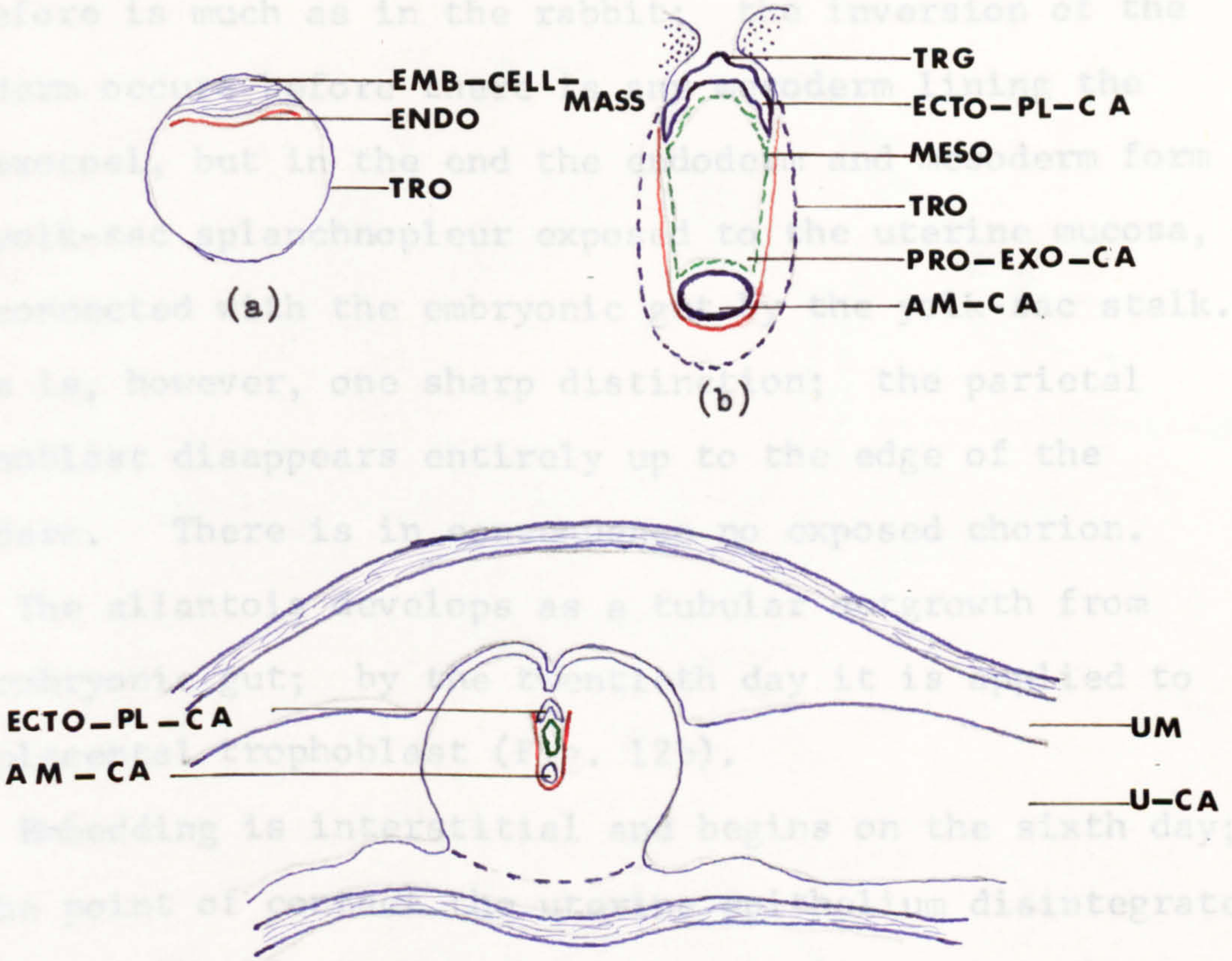


Figure 11

Blastocyst and implantation of guinea-pig

ENDO, endoderm; MESO, mesoderm; PRO-EXO-CA, pro-exocoelic cavity; TRO, trophoblast; UM, uterine muscle; for AM-CA, ECTO-PL-CA, EMB-CELL-MASS, TRG and U-CA see figure 6. (a is after Sansom and Hill, 1931; b and c are after Duval, 1892).

therefore is much as in the rabbit; the inversion of the endoderm occurs before there is any mesoderm lining the pro-exocoel, but in the end the endoderm and mesoderm form the yolk-sac splanchnopleur exposed to the uterine mucosa, and connected with the embryonic gut by the yolk-sac stalk. There is, however, one sharp distinction; the parietal trophoblast disappears entirely up to the edge of the endoderm. There is in consequence no exposed chorion.

The allantois develops as a tubular outgrowth from the embryonic gut; by the twentieth day it is applied to the placental trophoblast (Fig. 12b).

Embedding is interstitial and begins on the sixth day; at the point of contact the uterine epithelium disintegrates rapidly. The blastocyst then comes to lie within the uterine tissue (Fig. 11c). The epithelium at the margins of the implantation site regenerates until it finally covers the site. Entry of the blastocyst is at the abembryonic pole so that when completely implanted the ectoplacental trophoblast occupies a subepithelial position. As in the rat and mouse, so in the guinea-pig the uterine lumen becomes completely obliterated by the tenth day by the growth of the embryo and the fusion of the roof of the implantation cavity with the mesometrial mucosa where the surface epithelium has already disappeared. A new lumen reappears antimesometrially on the fifteenth day by the thinning of the uterine tissue over the expanding conceptus,

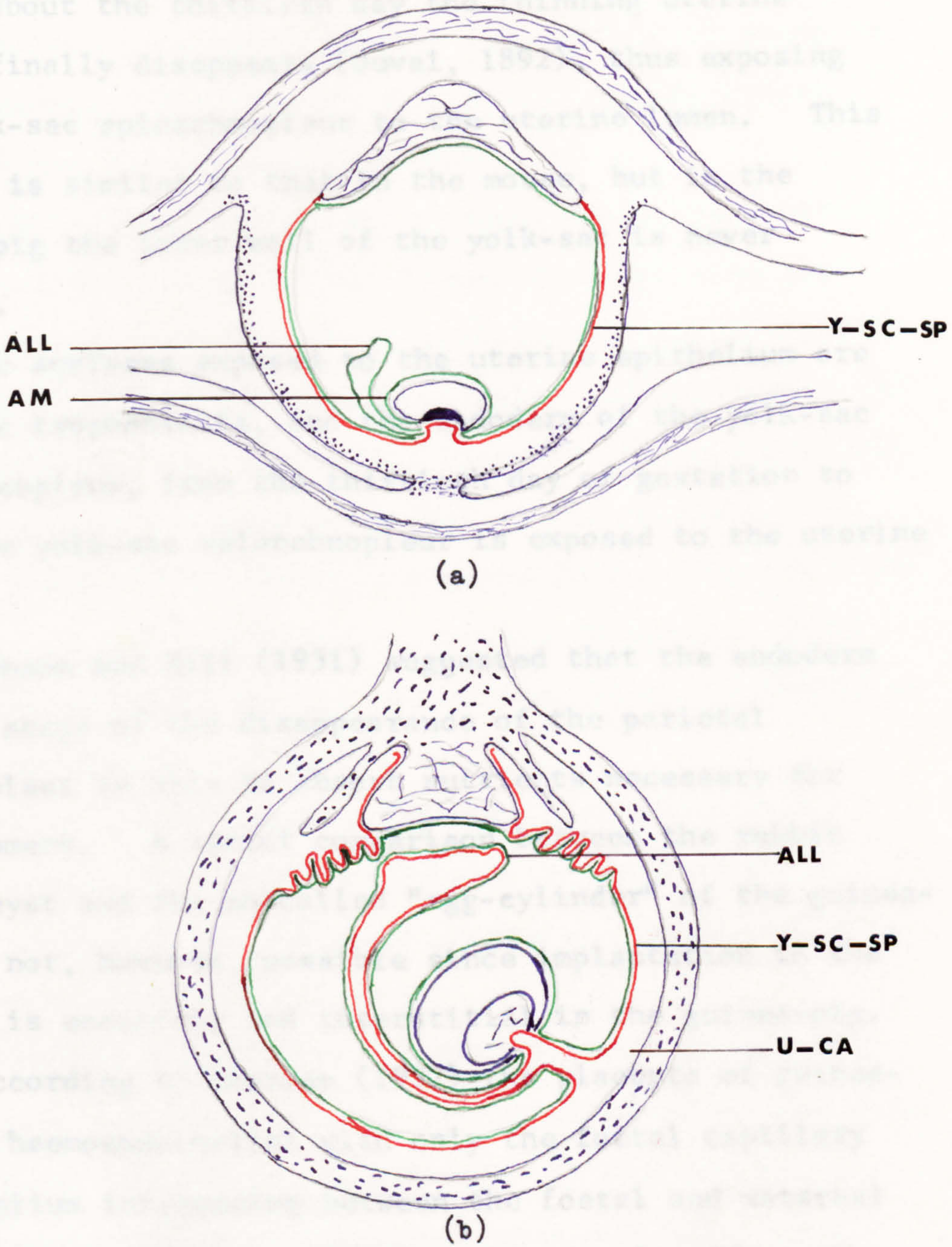


Figure 12

Embryonic membranes and decidua of guinea-pig

ALL, allantois; AM, amnion, U-CA, uterine cavity; Y-SC-SP, yolk-sac splanchnopleur. (a and b are after Mossman, 1937 with slight modification).

and by about the thirtieth day the thinning uterine tissue finally disappears (Duval, 1892), thus exposing the yolk-sac splanchnopleur to the uterine lumen. This process is similar to that in the mouse, but in the guinea-pig the lower wall of the yolk-sac is never present.

The surfaces exposed to the uterine epithelium are thus the trophoblasts, and the endoderm of the yolk-sac splanchnopleur, from the thirtieth day of gestation to term the yolk-sac splanchnopleur is exposed to the uterine lumen.

Sansom and Hill (1931) suggested that the endoderm at the stage of the disappearance of the parietal trophoblast is able to absorb nutrients necessary for development. A strict comparison between the rabbit blastocyst and the so-called "egg-cylinder" of the guinea-pig is not, however, possible since implantation in the rabbit is eccentric and interstitial in the guinea-pig.

According to Mossman (1937) the placenta of guinea-pig is haemoendothelial with only the foetal capillary endothelium intervening between the foetal and maternal circulation. Amoroso (1961), however, classifies the guinea-pig placenta as haemochorial with three layers intervening between foetal and maternal circulations. These are the trophoblast, foetal connective tissue and foetal capillary endothelium.

Transfer of immunity

Transfer of passive immunity from mother to young in the guinea-pig, like that of the rabbit, occurs before birth and not via colostrum or milk.

Staubli (1903) found that typhoid agglutinins passed from mother guinea-pig to her foetus in quantities that increased during the course of gestation. In other words the longer the time between immunization of the mother and birth the higher the agglutinin titre of foetal serum and the more nearly it approached that of the mother.

Anderson (1906) found that young guinea-pigs obtained a certain degree of resistance towards diphtheria toxin from their mothers when the mother was passively immunized with horse diphtheria antitoxin.

Ratner, Jackson and Gruehl (1927c,d) found that hypersensitivity to horse serum was transmitted from mother guinea-pig to her young in utero; there was no such transmission via the milk.

Schneider and Szathmary (1940) found that mother guinea-pigs immunized against diphtheria transferred their immunity to their young exclusively via the placenta. The antitoxin titre of the young serum was equal to or even greater than that of the mother. They also found that the antitoxin level of colostrum was substantially lower than that of the mother's serum.

Jo-Keiichiro (1953) found that complement fixing

antibodies to Rickettsia prowazeki and Rick. typhi were transmitted from mother guinea-pig to her young mainly through the placenta; no transmission of antibodies was demonstrated via colostrum or milk.

Barnes (1957) found that she could not detect antibodies in foetal sera of immune mothers before the 33rd day of gestation. From the 35th day of gestation until the 63rd day the ratio of foetal to maternal antibody titres increased from less than 0.1 per cent. to over 260 per cent. and then fell to 230 per cent. at birth.

Dancis, Shafron and Money (1957) found that all electrophoretically identifiable plasma proteins were transferred from the maternal to the foetal circulation.

Sclare and Taylor (1961) during their investigation on the relationship between thyroid auto-antibodies and thyroiditis studied the maternal and neonatal levels of auto-antibodies in guinea-pigs. They found that tanned-cell agglutinating auto-antibody titres in the newborn were close to maternal levels at or near delivery. In one instance, the titre of these antibodies in the newborn was higher than that of its mother.

Leissring and Anderson (1961a) found that in actively immunized pregnant guinea-pigs antibodies to Br. abortus were transferred to their foetuses as early as 35 days of gestation. The foetal to maternal concentration of antibodies increased from 1/16 at the 35th day to 1/2 or

1/1 at about the 50th day, and continued at this level until term.

Benacerraf, Ovary, Bloch and Franklin (1963) by electrophoresis separated two types of guinea-pig 7S antibodies to single protein antigens or hapten conjugates; these two types of antibodies, 7Sγ_1 and 7S γ_2, differred in their biological properties such as their capacity to induce anaphylaxis, cell lysis and opsonization. For example the 7Sγ_1 antibodies were capable of sensitizing guinea-pigs to passive systemic and cutaneous anaphylaxis, whereas 7Sγ_2 were not. Gamma 2 antibodies competed with γ_1 for the antigen and specifically inhibited passive cutaneous anaphylactic reactions provoked by γ_1. According to the authors, γ_2 antibodies, therefore, lacked the receptor for fixation to guinea-pig tissues and did not compete with γ_1 antibodies for receptor sites (Ovary, Benacerraf and Bloch, 1963). It was also found that 7Sγ_2, but not 7Sγ_1, antibodies were capable of fixing complement in vitro and of sensitizing antigen-coated erythrocytes for lysis in the presence of complement (Bloch, Kourilsky, Ovary and Benacerraf, 1963a).

The relative transmission of 7Sγ_1 and 7Sγ_2 guinea-pig antibodies from maternal to foetal circulation was investigated by Bloch, Kourilsky, Ovary and Benacerraf (1963b). They found that in actively or passively immunized guinea-pigs both types were transmitted to the

foetal circulation in significant amounts. They also found that milk contained low titres of γ_1 antibody compared to that of maternal sera collected simultaneously.

The route of transfer of antibodies from mother to foetus in the guinea-pig was investigated by Barnes (1957), who used Brambell's et al. (1949) technique of ligating the vitelline blood vessels. She showed that the route of transfer of antibodies to the foetus was, like that of the rabbit, via the vitelline blood vessels; little or no antibodies were absorbed from the foetal gut.

Leissring and Anderson (1961a) supported Barnes' finding in that the vitelline vessels were the major sites of entry of antibodies into the foetal circulation early in gestation, but they implicated the foetal gut as a major route at later stages of gestation.

The selectivity of transfer of antibodies to the foetal circulation is not thoroughly investigated. Hartley (1948, 51) showed that natural guinea-pig diphtheria antitoxin injected into guinea-pigs in late pregnancy was transferred to the foetus more readily than natural or "refined" horse diphtheria antitoxin or refined guinea-pig antitoxin in that order.

Kulangara and Schechtman (1963) by intravenous injections of human or bovine albumin and human γ-globulin into pregnant guinea-pigs found that all three proteins were present in the foetal sera in high titres. Traces

of these proteins were found in the amniotic fluid.

The formation of antibodies by newborn guinea-pigs was examined by Manzullo and Martino (1963). They injected diphtheria toxoid into newborn guinea-pigs at fifteen and thirty days of age. They found that at fifteen days the antibody forming mechanism of newborn guinea-pigs was fully mature. Antibodies were formed in spite of the presence of passively transferred antibodies from their immune mothers.

SUMMARY

1. Transfer of passive immunity from mother to young in the guinea-pig occurs before birth and not via colostrum or milk.
2. Antibody titre in the young increases with gestational age; it may reach levels equal to, or even higher than, that of the mother.
3. The route of antibody transfer to the foetus is, like that in the rabbit, from the maternal circulation, uterine cavity and vitelline circulation.
4. The selectivity of transfer of antibodies to the foetal circulation is not thoroughly investigated. A few experiments showed that natural antitoxin was transmitted to the foetus more readily than refined antitoxin.

MISCELLANEOUS

In monkeys transfer of globulin was found to be via the placenta and not via the amniotic fluid and foetal gut (Bangham, Hobbs and Terry, 1958; Bangham, 1960).

In dogs it was found that heterologous antitoxin injected into a pregnant bitch was absorbed by newborn puppies from the colostrum. (Mason, Dalling and Gordon, 1930). Transfer of passive immunity in dogs occurs to a small extent via the endotheliochorial placenta; puppies acquire their passive immunity mainly via the colostrum (Scheider and Szathmary, 1939b).

In cats, it was found that human and bovine albumins injected intravenously in their last week of pregnancy were transferred to the foetal blood in large amounts. However, human -globulin passed to the foetus in only small amounts even when relatively large doses were injected into the circulation of the mother (Kulangara and Schechtman, 1963).

EXPERIMENTAL METHODS

Hartley (1948) showed that in man the transfer of antibody from mother to young was selective in that pepsin-refined antiserum prepared in the horse and injected into the mother was not transferred to the foetus; this selectivity depended on the species of origin of the antibody molecule. Brambell and his colleagues (1949, 1950, 1951, 1952) showed that in rabbits antibodies were not transferred to the foetus through the placenta, but via the uterine cavity, yolk-sac splanchnopleur and vitelline circulation; this transfer was also selective in nature in that it depended on the species of origin of the antibody molecule and not on the molecular weight or size. The "refined" antibody molecule which had a smaller molecular weight than the original antibody was transferred less readily than the larger molecule from which it was derived (Brambell, Hemmings and Oakley, 1959).

The route of transfer of antibodies from mother to young in guinea-pig, like that in the rabbit, was found to be via the yolk-sac splanchnopleur and vitelline circulation (Barnes, 1957).

The present work was undertaken to study the transfer of antibodies from mother to young in the guinea-pig at various gestation periods and to find out whether this is selective.

MATERIAL

1. Animals

(a) Guinea-pigs.

All pregnant guinea-pigs used were of the Hartley strain. A few guinea-pigs of Mixed English strain were used for the production of anti-diphtheria and anti-tetanus antisera.

All animals were between seven and eight weeks of age and weighed between 350 and 400 g. when obtained from the livestock breeding station, Rayleigh, Essex. They were fed with diet S.G.I. supplemented with cabbage (4 oz. per animal per day) and ascorbic acid tablets (20 mg. per 100 ml. drinking water). Groups of six females were kept together in a 21 x 23 x 14 inch cage and were identified by ring numbers and a colour code.

(b) Rabbits

New Zealand white rabbits were used for immunization against tetanus. They were fed on diet S.G.I.

2. Diphtheria alum precipitated toxoid (A.P.T.)

Toxoid BA 2403A contained 1/10,000 Thiomersalate and was originally obtained from the Wellcome Research Laboratories, Langley Court, Beckenham, Kent.

3. Tetanus (A.P.T.)

Toxoid W6874 was also obtained from the Wellcome

Research Laboratories.

4. Diphtheria toxoid

This was preparation TMP 3064, used for immunization diluted 1 in 4 with broth.

5. Tetanus toxin

This preparation was dried toxin AW 331, originally obtained from the Wellcome Research Laboratories. The dried material was weighed and dissolved in broth to the concentration required.

6. Adjuvant

This preparation was a mixture of 8.5 ml of paraffin oil with 1.5 ml. arlacel. It was mixed with an equal volume of solution of AW 331.

7. Diphtheria toxin used for testing.

Preparation TP 976/8 was used. Its test dose at 0.02 unit was 1 ml. of a 1 in 1165 dilution in borate buffer saline.

8. Tetanus toxin used for testing.

Preparation TD 451, a free-dried tetanus toxin dissolved in 50 per cent. glycerol in saline was used. Its test dose at 0.01 units was 0.33 ml. of a 1 in 511 dilution in 5 per cent. broth in saline.

9. Clostridium welchii α-toxin

Preparation NX111 was used. 50 mg. of this preparation in 3.3 ml cagsal (Brooks, Sterne and Warrack, 1957) gave a solution of which one ml. contained the equivalent of 1 unit of α-antitoxin.

10. Standard Diphtheria antitoxin

This preparation was series LX 372; it contained 50 international units per ml.

11. Standard Tetanus antitoxin.

Two preparations were used as standard Tetanus antitoxin. Low level standard (LLS) contained 1.5 units per ml. LX 364 standard Tetanus antitoxin contained 0.20 units per ml.

12. Standard Clostridium welchii α-antitoxin.

Preparation R5609 was used. It contained 300 α-antitoxin units per ml.

13. International Standard Diphtheria Antitoxin.

One ml. of this antitoxin contained 10 International units; it was obtained from the Standard Laboratory, National Institute of Medical Research.

14. Phenol-ether.

This preparation was 50 per cent. (W/V) phenol in ether.

15. Saline-phenol-ether.

Half a millilitre of phenol-ether was diluted in 100 ml. saline. This preparation was used as diluent for serum samples if they had to be stored for more than a few days.

16. Saline

0.85 per cent. sodium chloride in distilled water.

17. Broth.

500 ml. of meat extract, 10 g. peptone and 5 g. NaCl were made up to one litre with distilled water.

18. Borate Buffer pH 8

The preparation was:

boric acid	69.44 g.,
sodium chloride	33.60 g.,
borax ($Na_2B_4O_7$)	45.86 g., made up to 8 litres

with distilled water, the pH was adjusted to eight with either acid or alkali.

19. "Cagsal"= Calcium-gelatin-saline

The solution was used as a diluent for Cl. welchii α-toxin. It was used to provide the necessary ions of calcium.

1 per cent. $CaCl_2$	138.75 ml.
5 per cent gelatin	100.00 ml.
NaCl	22.5 g.
phenol	2.5 g
sodium-thiomersalate	0.2 g.

These reagents were mixed and made up to 2.5 litres with distilled water. The solution was bottled in 100 ml. amounts, autoclaved at 125°C. for 10 minutes and stored at 4°C.

20. Sheep red blood cells.

Sheep blood was obtained from Leeds Slaughter House. To each 568 ml. of blood 100 ml. of preserving solution (see page 105) was added. The blood was washed twice with borate buffer of physiological saline; 1 part of preserved blood to 10 parts or buffer or saline. To each 1.6 ml. of packed cells 10 ml. of " diluent was added to give about 20 per cent. of cells. This preparation was standardized by Haldane Haemoglobinometer to give 20 per cent. of cells used in the present work.

The cells were standardized as follows:

From the preparation that contained bout 20 per cent. of cells in saline, a 1 per cent. solution of cells in water was prepared. Coal gas was passed for a few seconds into 2 ml. of the haemolysed cells and the resulting carboxyhaemoglobin was colour-matched with that of the Haldane Haemoglobinometer standard. Either cells or saline was added to the original cell suspension. Another 1 per cent. solution of cells in water was prepared and the process was repeated until colour-matched to that of the Haldane standard. The preparation of cells, 1 per cent. of which gave a colour-match with the standard,

contained 20 per cent. of cells used in the present work.

Solution for preservation of blood:-

This solution was used to preserve blood obtained from the Slaughter House.

Solution A: 22.5 g. sodium citrate in 750 ml. distilled water.

Solution B: 12.5 g. glucose in 250 ml. distilled water.

Each solution was steamed for 45 minutes and cooled. The two solutions were mixed and 5 ml. of 40 per cent. formalin was added.

It is interesting to note here that guinea-pig red cells may be used instead of sheep red cells. Guinea-pig blood was obtained by cardiac puncture from stock and defibrinated by gentle stirring with glass beads. The cells were standardized as described for sheep red cells.

21. Antisera

(a) Horse tetanus antitoxin

Preparation EX 919 contained 0.3 per cent. phenol as preservative; it had 300 units per ml. of tetanus antitoxin and 0.04 units per ml. of diphtheria antitoxin.

(b) Horse refined tetanus antitoxin

This preparation had 1500 units per ml. tetanus antitoxin and 3.0 units per ml. diphtheria antitoxin.

(c) Dog tetanus antiserum

Preparation EX 166/22.1.53 A/6 contained 18 units tetanus antitoxin per ml.

(d) Cow Cl. welchii α-antitoxin

Preparation EX 1369 (cow 7025/17.4.50) was used. It contained 300 units per ml.

(e) Human "A" tetanus antitoxin

This preparation was pooled human tetanus antiserum containing 50 units per ml. of tetanus antitoxin and 0.27 units per ml. of diphtheria antitoxin.

(f) Wilson's human tetanus antitoxin

This preparation contained 130 units per ml. of tetanus antitoxin and 0.3 units per ml. of diphtheria antitoxin.

(g) Saword's human tetanus antitoxin

This preparation contained 91 units per ml. of tetanus antitoxin and 0.22 units per ml. of diphtheria antitoxin.

(h) Rabbit diphtheria antitoxin

The preparations used were:

(i) Preparation 864/25.8.59 - contained 300 units per ml.

(ii) Preparation 828/10.3.59 - contained 80 units per ml.

(i) Rabbit tetanus antitoxin

Six preparations were used.

(i) Preparation 792/15.7.57 - contained 85 units per ml.

(ii) Preparation 791/15.7.57 - contained 38 units per ml.

(iii) Preparation 96/20.5.64 - contained 150 units per ml.

(iv) Preparation 95/20.5.64 - contained 300 units per ml.

(v) Preparation 93/20.5.64 - contained 235 units per ml.

(vi) Preparation 92/20.5.64 - contained 135 units per ml.

(j) Guinea-pig diphtheria and tetanus antitoxins

A number of these were used. Their values ranged between 30 and 135 units per ml. diphtheria antitoxin and between 36 and 220 units per ml. tetanus antitoxin.

(k) Guinea-pig normal serum

This was obtained from stock. Each sample usually contained less than 0.001 units per ml. of diphtheria antitoxin and less than 0.01 units per ml. of tetanus antitoxin.

22. Veterinary Nembutal.

This was obtained in sealed bottles and used according to directions (one ml. per five pounds body weight). Each ml. contained 60 mg. of Nembutal (sodium pentobarbitone), 10 per cent. alcohol and 20 per cent. propylene glycol.

23. Surgical instruments.

(a) Needles.

Size No. 11, half curved, round point. This was used for abdominal wall sutures.

Size No. 12, half curved, triangular point. This was used for skin sutures.

Size No. 26 GX ½ inch hypodermic needle. This was used for intra-uterine injections.

(b) Sutures.

Chinese twist silk No. 1 - used for abdominal wall sutures.

Plaited silk No. 12 - used for skin sutures. Both sutures were made by Pearsall's, England.

(c) Scissors and forceps

Six pairs of artery forceps

Two pairs of scissors

Two pairs of forceps

One pair of fine forceps

One pair of fine, curved artery forceps

(d) Surgical blades

All surgical instruments were boiled for half an hour before use, except the sutures and needles. These were sterilised at 125°C. (15 lb per sq. in.) for 15 minutes.

24. Polythene tubing of size 1 was used for feeding newborn guinea-pigs.

Preparation 2-13 inclusive and 21(a) - i (i) and (ii) inclusive were kindly supplied by Professor Oakley.

METHOD

1. Breeding

A stock of 60 - 80 females was maintained throughout. Six females were housed per cage 21 x 23 x 14 in. Two methods were followed to obtain guinea-pigs of known duration of pregnancy:

(a) vaginal smear.

(b) post-partum mating.

(a) Vaginal smear method

Stockard and Papanicolaou (1917) described the oestrous cycle in the guinea-pig; the dioestrous period is about sixteen days throughout the year, with insignificant variations during the different seasons. The period of sexual activity lasts for about twenty-four hours. This period can be identified by examination of the vaginal fluid for the changes occurring in the uterus and vagina. Four stages were described by these authors.

Stage 1, in which there is an abundance of vaginal squamous epithelial cells, staining grey with Harris' haematoxylin and eosin and showing many pycnotic nuclei. These cells were accompanied by cornified cells that stain red with Harris' haematoxylin and eosin.

Stage 2 was characterized by an increase of nucleated epithelial cells and by the cheesy appearance of the fluid.

Stage 3 was characterized by the appearance of leucocytes and the thinning consistency of the vaginal fluid.

Stage 4, in which slight haemorrhage began to appear, was not always observed.

Ovulation is spontaneous, occurring around the tenth hour after the beginning of heat (Asdell, 1946), near the end of the second stage or beginning of the third (Stockard and Papanicolaou, 1917). If fertilization did not occur within eight hours of ovulation, the chances

of eggs being fertilized fell.

During the early stages of the present work, vaginal smears of female guinea-pigs were obtained daily with a swab and were examined for stages described by Stockard and Papanicolaou (1917) after staining with Harris' haematoxylin and eosin. When the beginning of oestrous was observed the female was isolated and put in a separate cage with a competent male taken from a breeding stock. The female was kept with the male for twenty-four hours and then isolated in a cage and examined by palpation for pregnancy three to four weeks later. If she was then pregnant the duration of pregnancy was calculated by taking the day the female was put with the male as the first day. If she was not pregnant the female was returned to the stock and examined again for the next cycle.

It was later found that the swab method for obtaining the vaginal smear was not quite satisfactory, so it was changed to the pipette method described by Sell (1922). Pipettes were made from glass tube of fourteen cm. length and one cm. diameter, one end of which was drawn out to five mm. diameter. A twenty-five ml. capacity rubber bulb was attached to the wider end. One ml. of saline was flushed in and out of the vagina by means of this pipette, and the washings withdrawn. These were stained with Harris' haematoxylin and eosin after the film had been air-dried.

The method of using vaginal smears as indicator of oestrous and sexual receptivity of the female was found to be unfruitful. The same conclusion was drawn by Young (1937). A new method was later used in which each six females were put with one male in a cage. Those with broken vaginal membranes described by Stockard and Papanicolaou (1919) were examined for the presence of spermatozoa in the vagina. The vaginal contents were also examined by the pipette method. Females with positive samples were isolated on the same day and the time of pregnancy was counted from that day. This method proved more satisfactory and less time-consuming.

(b) Post-partum mating method.

According to Rowland (1949) oestrous in guinea-pigs follows parturition and is of short duration; mating takes place a few hours after parturition.

Post-partum breeding was used in the present work along with the vaginal smear method for the presence of spermatozoa. If certain matings were missed and no spermatozoa were observed in the smear and conception took place, the female was allowed to have her litter in the same cage and isolated some time after parturition. When the litter was born during the night or later in the evening, the female was allowed to stay the night with the male in the same cage and isolated the next morning. When the litter was born during the day, the female was

isolated at the end of the day. The isolated females were examined by palpation for pregnancy three to four weeks after parturition. If the female was pregnant, the duration of pregnancy was counted from the date of parturition as the first day. If the female was not pregnant she was returned to the original cage with the male after her young had been weaned, which usually occurs four weeks post-partum. By post-partum breeding a good number of successful pregnancies were obtained.

2. Immunization

(a) Immunization of guinea-pigs against diphtheria.

A total of 54 guinea-pigs were immunized against diphtheria toxoid. One ml. of BA 2403 A diluted 1 in 50 with saline was injected subcutaneously. Four weeks later another subcutaneous injection was made of one ml. BA 2403 A diluted 1 in 25 with saline. The animals were allowed to rest for six months. This period is very important to obtain avid sera. After the resting period, a series of subcutaneous injections were made. Diphtheria toxoid TMP 3064, diluted 1 in 4 with broth was injected three times a week. Each weekly series was given on three consecutive days, starting with 0.1 ml. and increasing 0.1 ml. per day to a maximum of 0.5 ml. The animals were allowed to rest for the remainder of the week. These weekly series were given until a high level of units was obtained. Test samples were obtained

by cardiac puncture, from animals taken at random. By this procedure, levels as high as 135 i.u. per ml. were obtained.

(b) Immunization of guinea-pigs against tetanus

A total of 52 guinea-pigs were immunized with tetanus toxoid. One ml. of APT 6874 A diluted 1 in 20 with saline was injected subcutaneously. Four weeks later another 1 ml. of APT 6874 A diluted 1 in 10 with saline was injected subcutaneously. Guinea-pigs were allowed to rest in order to obtain avid sera. After this period the animals were given two subcutaneous injections per week of increasing amounts of AW 331 (1 g. per 100 ml. broth) and an equal volume of adjuvant. Each dose was divided between two sites. The first dose was 0.1 ml. and this was increased to a maximum of 1 ml. in four weeks. The maximum amount was maintained until a high level of units was reached. By this procedure levels as high as 220 i.u. per ml. were obtained.

(c) Immunization of rabbits against tetanus.

Six rabbits were immunized with one ml. each of undiluted APT W6874 A administered subcutaneously and repeated after four weeks. After six months resting period the rabbits received increasing amounts of AW 331 (2 g. per 100 ml. broth) and an equal volume of adjuvant. On two days of each week the rabbits were given convenient volumes of this material, rising from 0.2 ml. at the

beginning to a maximum of 2.0 ml. divided between two sites. Blood samples were collected from each rabbit once a week before injection. By this procedure, values as high as 300 i.u. per ml. were obtained.

(d) Immunization of pregnant guinea-pigs

These animals were used to demonstrate the transfer of antibodies from mother to young at various gestation periods.

(i) Guinea-pigs that were used to demonstrate the transfer of diphtheria antitoxin received their first two doses as described under part (a) of this section. After six months of rest the females were mated post-partum. If pregnant they were started on TMP 3064 as described earlier. They were then killed at the gestation period required and samples were collected as described under "collection of samples". Foetal and maternal samples which were collected at term from some of these guinea-pigs were all taken after the rest period and before starting the series of injections with TMP 3064.

(ii) Guinea-pigs that were used to demonstrate the transfer of tetanus antitoxin received their first two doses as described under part (b) of this section. After six months of rest the females were mated post-partum. If pregnant they were started on the weekly series of injections of AW 331 as described earlier. Foetal and maternal blood samples taken at term were all collected

after the rest period and before the weekly series of AW 331 were made.

3. Intracardiac injections

All intracardiac punctures were performed under ether anaesthesia. Intracardiac injections of test antisera were made by first inserting the needle into the heart, and drawing a blood sample into the syringe before and after the material was injected. The material was administered slowly over 10 seconds.

4. Intra-uterine injections of antisera

To test the selectivity of foetal membranes, intra-uterine injections into non-immune pregnant guinea-pigs were made in order to expose the foetal membranes directly to the antibodies under test. In this way passage of antibodies through the maternal tissues to the uterine lumen was avoided. If there was selection during this passage, then it was avoided. Also, to avoid any dilution by maternal blood.

Operations were performed under aseptic conditions; injections were made into one gravid horn, leaving the other as a control. Pregnant guinea-pigs at various gestation periods received various mixtures of antitoxins - usually of homologous antiserum plus one or two heterologous sera or a mixture of natural and refined antitoxins. The animal was starved eighteen to twenty-four hours prior to the operation to allow emptying of the intestinal

tract. The skin was clipped on the ventral side and a dose of anaesthetic (Nembutal) was administered intraperitoneally. It is very necessary to administer the right amount, otherwise abortion ensues. If the dose was not sufficient then it was supplemented with a mixture of air and ether pumped by a small device made for that purpose. A plastic cone filled with cotton wool, was applied to the animal's snout and an ether-air mixture was pumped through. A median-ventral incision was made followed by another incision in the abdominal wall. The two layers of skin were then clipped with three artery forceps on each side and the gravid uterine horn was exteriorized. A piece of plaited-silk No. 1 was passed under and around the tubal end of the horn with the help of an assistant. A loose half knot was made and the mixture was injected from a 2 ml. syringe at a point above the knot. After injection of the right amount (0.5 ml. of mixture per foetus) the half knot was tightened and the reef knot completed. By tying the horn below the injection point, leakage of material from the horn was avoided. The gravid uterus was then returned to the abdomen by allowing the foetuses to slide slowly into position. This stage was very critical because if the foetuses were forced inside, abortion often ensued. After the foetuses had taken their position inside the abdomen, the abdominal wall was sutured with size one silk.

The skin was then sutured with size twelve silk, and the animal was put in a warm room.

Twenty-four hours later the animal was killed with an overdose of anaesthetic. The abdomen was opened and samples were collected as described under "collection of samples".

5. Feeding of newborn guinea-pigs with antisera.

Newborn guinea-pigs from non-immune mothers were fed with various antitoxins, starting from two to twenty-four hours of age. The young were starved two to three hours before administration of serum and weighed. Size one polythene tubing was connected, by a small flame, to a 21 gauge needle attached to a tuberculin syringe, into which the material had previously been drawn. The free end of the tubing was inserted slowly and carefully into the stomach of the young. A known amount of antiserum was administered and the young were returned to their mother.

Twenty-four hours later the young were killed under ether anaesthesia and were bled directly from the heart. Wherever possible one animal from the same litter, not fed with antiserum, was used as a control.

6. Collection of samples

(a) Immune guinea-pigs and their young

Immune guinea-pigs were killed with an overdose of Nembutal at various gestation periods. The abdominal

wall was clipped and the gravid uteri were exteriorized through median ventral abdominal incision. The foetuses were exposed by tearing the uterine wall with two fine forceps, avaoiding excessive bleeding. Samples were collected from the foetuses in order starting from the foetus nearest the fimbriated end of the horn. The yolk-sac was usually removed; no samples were obtained from its cavity since the fluid was less than required for a single test. The amnion was punctured with a Pasteur pipette and the amniotic fluid was collected, centrifuged and kept in sterile bottles without preservative. The foetus was then laid on its back while still attached to the placenta and two parasternal incisions were made in its chest. The tip of a fine, curved artery forceps was inserted through these incisions and the skin was cut below the part that was clipped with the forceps to expose the heart. The pericardium was removed with fine forceps and blood was collected directly from the heart with a Pasteur pipette. To obtain more blood, the foetal liver was usually squeezed between two fingers, forcing the blood to flow through the hepatic vein into the posterior vena cava. Stomach samples were obtained by cutting the ventral abdominal wall and exposing the stomach (which usually lies under the liver), puncturing it with a Pasteur pipette and collecting the contents. Samples contaminated with blood were usually discarded. Those

obtained successfully were put in clean, sterile tubes without preservative.

Maternal blood samples were collected by cardiac puncture. Maternal and foetal blood were treated as described under "blood samples".

Newborn animals were killed with ether and blood samples taken from the exposed heart with a Pasteur pipette.

Samples from ground foetuses were obtained by first ligating the umbilical cord close to the placenta, and then cutting it. Foetuses and membranes were ground with pestle and mortar without the use of sand. The material was transferred to a centrifuge tube and centrifuged. The supernatant fluid was obtained and the samples were preserved with phenol-ether. Each sample was assayed and the number of units per ml. serum were calculated as described under "results".

(b) Blood from immune guinea-pigs

The animals were anaesthetised and bled out from the severed artery.

(c) Blood from immune rabbits

These samples were collected by puncturing the marginal ear vein and allowing the blood to drip into sterile boiling-tube.

7. Blood samples

All blood samples, including those of foetuses, were allowed to stand at room temperature and the clot was

separated from the sides of the tube with a fine glass rod. The samples were then left overnight in the refrigerator for the clot to retract. The next morning, the samples were left for less than two hours at room temperature to allow the serum to separate. The clots were centrifuged off and the sera were kept in sterile tubes without any preservative. Those samples not collected under aseptic conditions were always filtered through Oxoid membrane filter.

8. Pepsin digestion of antibody

An immune serum to a certain antigen is composed of a heterogeneous population of antibodies. These may differ in physical or immunochemical properties or both (Kabat and Meyer's Experimental Immunochemistry, 1961).

An antibody molecule has two properties. One is the capacity to react with the specific antigen and the other is the capacity to mediate a variety of biological phenomena, such as cell lysis, opsonization or passage through biological membranes. These properties have been assigned to two different types of polypeptide chains of the γ-globulin molecule (Edelman and Benacerraf, 1962).

The property of passage through biological membranes is of great interest. It depends not only on the type of molecule itself, but also on the type of membrane. Certain membranes, such as the gut of newborn calf, pig and sheep, allow unselective passage of antibodies within

the first few hours of prenatal life, whilst the rabbit's yolk-sac splanchnopleur is selective in its admission of antibodies into the foetal circulation. The selectivity of the yolk-sac splanchnopleur of foetal rabbits does not depend on molecular size or weight of the antibody molecule, but on the species of origin. Pepsin-digested fragments pass to the foetal rabbit less readily than the unmodified molecule. Porter (1959) hydrolysed rabbit γ-globulin with crystalline papain and obtained three fragments. Fragments I and II had a molecular weight of 50-55,000 and retained antibody activity in the sense that they combine with the specific antigen. Fragment III had a molecular weight of 80,000 and much of the antigenic specificity of the original molecule, but no antibody activity; it is probably the fragment that is destroyed by pepsin digestion and heating.

Part of the present work was to compare the permeability of the guinea-pig foetal membranes to pepsin-digested homologous antitoxin with that to the unmodified γ-globulin.

Procedure.

The method followed was that of Pope as modified by Harms (1948). Guinea-pig antitetanus antiserum containing 90 i.u. per ml. was diluted with an equal volume of tap water and the pH adjusted to 3.5 with 1 per cent. concentrated HCl. Pepsin was added to 0.5

per cent., the pH was adjusted to 3.2 and digestion was carried out at room temperature (20°C) for two hours. The pH was readjusted to 7.4 and ammonium sulphate was added to 30 per cent. The precipitate was centrifuged and dialysed overnight against running water. The material was reconstituted to half the original volume and filtered through oxoid membrane filters. The yield of antitoxin was about 50 per cent.

9. Diphtheria antitoxin titration:

The method of Römer and Sames (1909) as modified by Glenny and Llewellyn-Jones (1931) was followed. When diphtheria toxin is injected into the depilated guinea-pig skin it produces a local erythema the size of which varies with the amount of toxin injected; larger amounts of toxin may produce necrosis. The principle of estimation of the number of units of diphtheria antitoxin of a sample is that various amounts of the unknown sample are mixed with a constant amount of toxin (test dose) and the neutralizing effect of the antitoxin to the toxin is read after injection into the guinea-pig skin. It is necessary to inject the mixture intradermally into an albino guinea-pig because the erythema produced is difficult to read in a coloured guinea-pig skin.

The test dose of a toxin at one unit may be defined as that amount of toxin which when mixed with one unit of standard antitoxin produces a certain effect on the

indicator; erythema of 5 mm. diameter in the guinea-pig skin in this case. To determine the test dose at one unit various amounts ot toxin that differ from each other by one logarithmic volume were put in a series of clean, dry tubes and to each was added one unit of diphtheria standard antitoxin contained in one ml. The mixtures were made up to 20 ml. with borate buffer and were allowed to stand at room temperature for at least half-an-hour. Two tenths of a ml. from each mixture was injected into the depilated albino guinea-pig skin and the reaction was read after 48 hr. The actual amount of toxin that was injected intradermally into the skin was 0.01 of the test dose at one unit.

Estimation of the number of units of diphtheria antitoxin in a sample was usually made by first guessing how many units one would expect in that sample. If the expected value was less than 0.1 unit the "capillary" method was followed; if it was more than 0.1 unit, other methods were followed.

<u>"Capillary" method:</u>

The test dose determined at one unit was arranged to be in one ml. The following dilutions were prepared of the test dose that one ml. contained 1/10, 1/12, 1/15, 1/20, 1/25, 1/30, 1/40, 1/50, 1/60, 1/80 and 1/100 of a unit. Lower values may be prepared as required; however the lowest value that can be tested for is

0.0005 unit.

With a specially calibrated capillary pipette 0.12 ml. of the toxin dilution was pipetted into a clean, dry tube, an equal volume of diluted or undiluted antitoxin (unknown sample in this case) was added to each tube. The toxin-antitoxin mixture was shaken gently, and incubated at room temperature for at least half-an-hour. Each mixture was then injected intradermally into the depilated albino guinea-pig skin. The reaction on the guinea-pig skin was read after 24 and 48 hr. taking the second reading as the final result. The end point was a slight redness of few mm. diameter in the guinea-pig skin. The number of units of diphtheria antitoxin in the sample giving a slight reaction was calculated from the number of units of the toxin with which it was mixed before injection.

Example (1)

If the value of an unknown sample was expected to be between 0.04 and 0.1 units of diphtheria antitoxin, then only five dilutions of toxin were prepared. Each dilution of toxin differed in value from the one preceding or following it by approximately 20 per cent. In this case dilutions of 1/10, 1/12, 1/15, 1/20 and 1/25 units of the test dose were prepared; each toxin dilution was to be contained in 1 ml. Each toxin dilution was mixed with an equal volume of diluted or undiluted antitoxin and the

mixtures were treated as described above. The test was repeated until two consecutive results agreed with each other within the limits of the test as follows:

Mix injected at toxin level	reaction after 48 hrs 1st test	2nd test
1/25	-	-
1/20	-	-
1/15	s	±
1/12	±	+
1/10	+	+

The value of antitoxin was in this example 1/15 which is equal to 0.067 units per ml.

Other methods:

These were used when the antitoxin value was expected to be above 0.1 units of diphtheria antitoxin.

When the antitoxin value was expected to be between 0.1 and 1.0 i.u./ml. the following procedure was followed:

The test dose at one unit was arranged to be contained in one ml. From this preparation 1:50 dilution was prepared and 0.25 ml was put in a series of clean, dry tubes (0.25 ml is equivalent to 1:200 of a test dose at one unit). Various amounts of diluted antitoxin were added to each tube, the contents were mixed and incubated at room temperature. Two tenths of a milliliter of each mixture was then injected intradermally in the depilated guinea-pig skin and the reaction was read 24 and 48 hr

later. The number of units of the sample was then calculated from the dilution that gave a slight reaction in the guinea-pig skin.

Example (2)

If the value of an unknown sample was expected to be between 0.1 and 0.5 units, then the test dose at 1:200 unit was put in three clean, dry tubes. The sample was diluted 1:20 with borate buffer and to each tube the following amounts were added to the test dose

Tube No.	Units expected	Ml. dil. sample	reaction after 48 hrs
1	0.1	1.0	-
2	0.2	0.5	-
3	0.5	0.2	+

From the above result the sample value was found to be between 0.2 and 0.5 units. Another test was made using the test dose of toxin at 1:200 of a unit as above and diluting the sample to 1:40 considering it to have 0.2 units. Various amounts of the sample dilutions were then added to one test dose at 1:200 of a unit. The mixtures were treated as above and the reaction read after 48 hr. The amounts of antitoxin dilution added to each test dose differed from each other by about 20 per cent., as:

No. of units expected	ml. dil. antitoxin	reaction
0.20	1.0	-
0.24	0.83	-
0.30	0.67	±
0.36	0.56	+
0.45	0.45	+

The above result showed that the value of the sample was between 0.24 and 0.36 units. Another test was made as above but using the antitoxin dilution 1:48 in amounts that differ by 10 per cent. from each other as follows:

No. of units expected	ml. dil. antitoxin	reaction (1)
0.24	1.0	-
0.27	0.89	S
0.30	0.8	±
0.33	0.73	+
0.36	0.67	+

Therefore the value of the sample was considered to be 0.30 units/ml.

Another test was made as just described in the final estimation to confirm the value as follows:

No. of units expected	ml. dil. antitoxin	reaction (2)
0.24	1.0	-
0.27	0.89	-
0.30	0.8	s^{+}_{-}
0.33	0.73	+
0.36	0.67	+

Example (3)

If the value of an unknown sample was expected to be more than one unit, then the titration was as follows:

Suppose the value was expected to be 36 units per ml. Then the antitoxin was diluted to a solution containing 1:50th unit per ml. to allow for the antitoxin having a value 20 per cent. lower than the expected value; that is 30 instead of 36 units per ml. in this example. Therefore the antitoxin was diluted 1:1500 in borate buffer.

The toxin was prepared so that the test dose at 1:50th of a unit was contained in 1 ml. One ml. of the diluted toxin was then put in a series of tubes. Various amounts of the diluted antitoxin were then added to each tube giving a range of 30 - 45 units in this example; in other words the expected value $\pm$ 20 per cent. Thus to one ml. of the diluted toxin the following amounts of the diluted antitoxin were added:

units expected	ml. antitoxin	reaction (1)
30	1.0	-
33	0.91	-
36	0.83	s
40	0.75	±
45	0.67	+

The test was repeated and another set of reaction was obtained as follows:

units expected	ml. antitoxin	reaction (2)
30	1.0	-
33	0.91	-
36	0.83	±
40	0.75	+
45	0.67	+

The value of the antitoxin was therefore considered to be 36 units as suggested.

With each diphtheria testing two controls were prepared.

(1) Toxin control:

The test dose determined at one unit was arranged to be in one ml. A dilution of 1:2000 and 1:4000 units of toxin were prepared. By capillary method equal volumes of these two dilutions were mixed with borate buffer and injected into the guinea-pig skin as described.

The expected result in this control was that no reaction or only a slight one occurred at toxin dilution

equivalent to 1/4000 of a unit and a slight but well defined reaction occurred at a toxin dilution equivalent to 1/2000 of a unit. If there was no reaction in the skin at the equivalent of 1/2000 of a unit on repeated tests, then the test dose was re-estimated at one unit.

(2) Standard control:

This was prepared by using a standard diphtheria antitoxin obtained from Professor Oakley.

One ml. of the test dose at 1:50th of a unit was put into each of five clean, dry tubes. The standard antitoxin was diluted so that it would have contained 1:50th of a unit if its value were 20 per cent. less than its correct value. For example: if the standard antitoxin had a value of 40 units per ml. then it was considered to have a value of 33 unit per ml. A dilution of 1:1650 in borate buffer was prepared so that each ml. of this preparation contained 1:50th of a unit of antitoxin. Various amounts of the diluted antitoxin were then added to each of the above tubes. These amounts differ by 10 per cent. from each other as follows:

Units	ml. diluted antitoxin	reaction (1)
33	1.0	-
36	0.91	-
40	0.82	±
45	0.73	+
50	0.66	+

1C. Tetanus antitoxin titration

When tetanus toxin is injected into mice it either kills them or produces various signs of tetanus, depending on the amount and potency of the toxin. When tetanus antitoxin is mixed with the toxin before injection into mice and the mixture is allowed to react under standard conditions the effect of tetanus toxin on mice is greatly reduced or completely neutralized. The principle of the tetanus antitoxin titration is that a constant amount of toxin is allowed to react with a series of various amounts of antitoxin, each mixture is injected into one mouse and the degree of protection is calculated. The method followed was that of Glenny and Stevens (1938).

Tetanus test dose used in the present work was that amount of toxin which when mixed with 0.01 International unit of tetanus antitoxin produced death in mice between 72 and 96 hrs.; this was the test dose at one hundredth of a unit. When this test dose was diluted 10 times and then mixed with 0.001 International unit of tetanus antitoxin it also produced death in mice at 72 to 96 hrs; this was called the test dose at one thousandth of a unit.

To estimate the test dose accurately, a series of various amounts of toxin diluted in broth-saline (90 per cent. saline and 10 per cent. broth) were mixed with either 0.01 or 0.001 International unit of antitoxin

depending on whether the test dose was estimated at one hundredth or one thousandth of a unit as in the above definitions. The difference between each amount of toxin was one logarithmic volume. The toxin-antitoxin mixtures were shaken gently and incubated at room temperature for at least one hour. Each mixture was then injected subcutaneously in the thigh region of a mouse. The mice used in this work were 20-30 g. in weight and of the same strain. Readings were made after 24, 48, 72 and 96 hr and signs of tetanus were recorded as follows:

(1) A negative reaction (-) meant that the mouse was normal at reading.

(2) A slight reaction (SL) meant that the mouse was lifting the injected leg heavily. This was the first sign of positive tetanus.

(3) A slight tetanus reaction (SLT) meant that the muscle of the thigh was becoming stiff and of little use for walking. Also the tail might be slightly stiff.

(4) A reaction of tetanus (T) meant that the signs of tetanus were more pronounced. The injected leg was very stiff and the spine was twisted. The second leg might also be affected at this stage.

(5) A double tetanus (TT) meant that the mouse was lying on its back, and helpless. At this stage the mouse must be killed because it can no longer feed or drink.

(6) When the mouse was found dead at reading it was

recorded as (†).

A mouse may show various signs of tetanus during 96 hr such as (SL) during the first 24 hours., (SLT) during the next 24 hrs. and (TT) during the following day.

Example.

	time in hours			
mouse	24	48	72	96
1	-	SL	SL	SLT
2	-	SL	SLT	T
3	SL	SLT	TT	
4	SL	SLT	†	
5	SLT	TT		

The end point was a double tetanus (TT) at the 72-hr reading (mouse No. 3 in the above example).

To estimate the value of a sample containing unknown number of units of tetanus antitoxin the following procedure was used:-

The test dose was pipetted into series of clean, dry tubes. Various amounts of the unknown sample, diluted or undiluted, were added to one tube each. These volumes differ from each other by 10 per cent. The mixtures were then treated as described under the estimation of the test dose.

It must be remembered that the mouse is a small animal and the volumes injected should not exceed one ml per mouse. Hence it was preferable to make the antitoxin

dilutions twice as concentrated thus using half the necessary volume.

Example (1)

When the sample under test was expected to have a value more than 0.1 unit (between 0.36 and 0.55 units for test given below), the test was carried out using a test dose estimated at 0.01 of a unit. The toxin used in the present work had a test dose of 0.33 ml at 0.01 of a unit. The antitoxin sample under test was then diluted 1:18 and various amounts of this dilution were added to the test dose as follows:

Sample value tested for	ml. test dose at 0.01 unit	ml. of diluted sample	mouse
0.36	0.33	0.5	1
0.4	0.33	0.45	2
0.45	0.33	0.4	3
0.5	0.33	0.36	4
0.55	0.33	0.33	5

Results.

Mouse	reaction at hrs. 24	48	72	96
1	-	SL	SLT	SLT
2	-	SLT	T	TT
3	SL	SLT	TT	
4	SLT	T	†	
5	T	†		

Example (2)

When the sample under test was expected to have a

value less than C.1 unit (for the example given below expected value was between 0.012 and 0.018). The test dose at 0.001 of a unit was put in a series of 5 tubes. The sample under test was diluted 1:6 and the following amounts were added to the test dose:

serum value tested for	ml test dose at 0.001 unit	ml of diluted sample	mouse
0.0120	0.33	0.5	1
0.0135	0.33	0.44	2
0.0150	0.33	0.4	3
0.0165	0.33	0.36	4
0.0180	0.33	0.33	5

Results

Mouse	reaction at hrs 24	48	72	96
1	-	-	SL	SL
2	-	SL	SLT	SLT
3	-	SLT	TT	
4	SL	SLT	†	
5	SLT	T	†	

A control test was always carried with the samples by mixing various amounts of standard antitoxin with the test dose, either at 0.01 or 0.001 of a unit as required.

Example (3)

The standard antitoxin used had a value of 1.5 i.u/ml. In this example, the test was carried at 0.01 of a unit. The standard antitoxin was therefore diluted 1:60 and the following amounts were added to the test dose:

i.u/ml serum value	ml. of dil. std.	mouse
1.2	0.5	1
1.35	0.45	2
1.5	0.4	3
1.65	0.36	4
1.8	0.33	5

Results.

	reaction at hrs			
Mouse	24	48	72	96
1	-	SL	SL	SLT
2	-	SL	SLT	T
3	SL	SLT	TT	
4	SLT	T	†	
5	SLT	†		

The majority of the foetal sera and amniotic fluid samples were tested at 0.001 of a unit since their values were very low. It was therefore necessary to test the serum to which the foetuses were exposed at the same level in order to keep the values comparable.

11. "Capillary" method of Cl. welchii α-antitoxin titration

The method followed was that described by Professor Oakley (unpublished)

(a) Determination of the haemolytic test dose of Cl. welchii α-toxin:

The α-toxin produced by Cl. welchii Type C is an enzyme (lecithinase); it is lethal to mice, dermonecrotic to guinea-pig and haemolytic to the red cells of most

laboratory animals except horses and goats (Oakley, 1954). Its haemolytic activity occurs only in the presence of ionised calcium (MacFarlane, Oakley and Anderson, 1941; Oakley and Warrack, 1941).

Various dilutions of toxin in cagsal were prepared, each differed from the one preceding or following it by about 10 of 20 per cent. A standard Cl. welchii α-antitoxin was diluted so that it contained 1 unit of α-antitoxin per ml. With special "capillary" pipette 0.12 ml. of toxin dilution was put in flat-bottomed agglutination tubes specially prepared for the purpose. The pipette was washed with hot water and dried. An equal volume of the diluted standard α-antitoxin was added to each tube. The contents were mixed by tapping and were allowed to react at room temperature for 30 minutes. Five hundredth of a millilitre of 10 per cent. sheep red blood cells in saline or in borate buffer were added to each tube. The contents were mixed by tapping and were incubated in a water bath at 37°C. for one hr. The tubes were left at room temperature and were first read few hrs after they were removed from the bath. A second reading was made after 24 hr. The tube that showed slight haemolysis of the red cells was the one that contained the toxin dilution equivalent to 1 unit.

(b) Determination of antitoxic value of samples.

The test dose of Cl. welchii α-toxin was diluted

in cagsal as for diphtheria capillary testing to give 1, 1/2, 1/5, 1/10, 1/25, 1/50, 1/100 unit per ml. Toxin might be diluted at 10 per cent. intervals as required. Equal volumes of toxin were mixed with the diluted or undiluted samples and treated as described under determination of test dose. The numbers of units that was contained per ml. of sample could be calculated from that tube that gave slight haemolysis of the red cells after 24 hr. or best after few hrs. at room temperature.

(c) Controls.

(i) Toxin control:- Toxin was diluted in cagsal so that one ml. contained the equivalent of 1/100, 1/250, 1/500 and 1/1000 unit. By capillary pipette one volume of toxin was mixed with an equal volume of borate buffer or diluted or undiluted normal guinea-pig serum. Half a volume of red cells were added, the contents were mixed by tapping and were incubated at 37°C. as described earlier.

(ii) Standard antitoxin control:- with each test a standard Cl. welchii α-antitoxin was diluted as required. Equal volumes of toxin dilution was mixed with diluted antitoxin. The tubes that contained equal number of units of standard antitoxin and of test dose dilution should give a slight haemolysis of red cells. Thus a standard antitoxin might be diluted to contain 1/250 units per ml. Toxin was diluted in cagsal to contain the equivalent of 1/400, 1/300, 1/250 and 1/200 of a unit

per ml. Equal volumes of each toxin dilution was mixed with one volume of diluted standard antitoxin and the mixtures were treated as described earlier.

The results in this case should be as follows:-

Mix. at toxin level	reaction after 24 hrs.
1/400	-
1/300	-
1/250	±
1/200	+

RESULTS

(1) Transfer of antibody from actively immunized mothers to their foetuses:

Eleven pregnant guinea-pigs actively immunized against diphtheria were killed from thirty days of gestation to full term (about sixty-five days). Samples of maternal and foetal blood and amniotic fluids were collected at killing and the serum values, expressed in international units per ml. (i.u./ml.), are presented in table 1. The antibody concentration in foetal serum samples and amniotic fluids was also expressed as a concentration quotient (C.Q., Batty et al. 1954), defined as the ratio of the antitoxin concentration of the sample to that of the serum to which it was exposed i.e., maternal serum in this case.

Because it was not possible to obtain a serum sample sufficient for testing from a foetus of thirty days of age, the foetus was ground with its membranes and the result was compared with that of an older age where sera of litter mates were assayed individually (guinea-pig 365 1R and 354 1L). The average C.Q. value for sera of five litter mates at forty-two days of age, of which the sixth foetus was ground was 0.113. The C.Q. of the total antitoxin transferred to the single conceptus at forty-two days was 0.045 and that at thirty days was 0.01; the

Transfer of antibody (diphtheria antitoxin) from their

Table

Ref No.		Preg. (days)	Mat. ser. (i.u./ml.)(1)	Foet. ser. (i.u./ml.)
362	1L	30	65	-
	2L			-
365	1R	30	65	0.65(3)
359	1R	39	33	2.9
	2R			3.8
	3R			2.4
	1L			3.6
	2L			3.6
	3L			3.3
354	1R	42	48	4.8
	2R			5.0
	1L			2.2(3)
	2L			6.2
	3L			6.5
	4L			4.8
368	1R	47	90	18
	2R			23
	3R			18
	1L			17.5
	2L			21
	3L			23

the circulation of actively immune guinea-pigs to foetuses

I

C.Q. (2)	Amn. fluid (i.u./ml.)	C.Q.
	< 0.1	< 0.0015
	< 0.1	< 0.0015
0.025[3]	-	
0.087	0.010	0.00033
0.115	0.014	0.00042
0.073	0.011	0.000334
0.109	0.014	0.00042
0.109	0.011	0.000334
0.100	0.014	0.00042
0.100	0.025	0.00052
0.104	0.025	0.00052
	-	
0.129	0.025	0.00052
0.135	0.025	0.00052
0.100	0.025	0.00052
0.200	0.165	0.00119
0.255	0.2	0.00222
0.200	0.24	0.00267
0.19	0.1	0.00111
0.233	0.135	0.00150
0.255	0.12	0.00134

Table I

Ref. No.		Preg. (days)	Mat. ser. (i.u./ml.)(1)	Foet. ser. (i.u./ml.)
363	1R	50	50	16.5
	2R			19
	3R			22
	4R			18
	1L			20
168	1R	54	100	37
	2R			36
	1L			33
	2L			45
369	1R	60	90	68
	2R			85
	3R			85
	4R			60
	1L			68
365	1	Term (- 65)	0.47	0.57
356	1	Term	1.7	2.4
	2			2.3
737	1	Term	2.2	6.5
	2			6.5

(1) International units per ml.

(3) Ground foetus plus amniotic fluid (see text for details)

(Continued)

C.Q. (2)	Amn.fluid (i.u./ml.)	C.Q.
C.33	C.12	C.CC24
C.38	C.15	C.CC3C
C.44	C.12	C.CC24
C.36	C.5(4)	C.C1CC
C.4C	C.13	C.CC2C
C.37	C.22	C.CC22
C.36	1.6(4)	C.C16
C.33	C.17	C.CC17
C.45	C.22	C.CC22
C.75	C.1	C.CC1
C.94	C.33	C.CC36
C.94	C.4	C.CC43
C.67	C.36	C.CC4
C.75	C.11	C.CC12
1.2	-	-
1.3	-	-
1.3	-	-
2.95	-	-
2.95	-	-

(2) Concentration of units in fluid to that of the maternal serum

(4) May be contaminated with blood

- = No sample

approximate C.Q. for foetal serum at thirty days of age would be 0.025 if it is assumed that the increase in blood volume is proportional to the increase in weight during the twelve days of age difference. In another experiment at thirty days of age only the amniotic fluid was collected (guinea-pig 362 1L and 2L).

Table 1 shows that the C.Q. of foetal sera increases from about 0.025 at thirty days to 2.95 at full term. There is a gradual increase in the concentration of antitoxin from thirty to sixty days of gestation. A sudden increase in the concentration of antitoxin in the foetal sera occur during the last few days before term. The C.Q. for foetal sera rose from 0.67 (the lowest value) at 60 days, to 2.95 (the highest value) at term. This meant that the concentration of antitoxin during 60 days of gestation was increased to two to four times during the few days before term. These results showed the ability of the foetus to concentrate antibodies against a concentration gradient.

The concentration of diphtheria antitoxin in the amniotic fluid was much lower than that in the foetal serum at all stages of gestation tested (table 1). Like that of the foetal sera, the antitoxin value of the amniotic fluid appeared to rise as gestation advanced. There was about sixfold increase in the C.Q. for amniotic fluid between thirty-nine days and fifty-four days of

gestation. The amount of amniotic fluid at thirty days was insufficient for me to carry the test to finality; therefore, the C.Q. value was expressed as less than the lowest value tested. Two amniotic fluid samples (guinea-pig 363 4R and 168 2R) had much higher antitoxin concentration than those of their litter mates. This high concentration might have been due to contamination with blood.

The transfer of tetanus antitoxin to the foetus was studied as well as that for diphtheria antitoxin. Eight guinea-pigs immunized against tetanus were killed from thirty days of gestation to full term. Foetal and maternal blood and amniotic fluid were collected at killing. The samples were assayed and their values were expressed as i.u. per ml. and the C.Q. values were determined (table 2). Blood samples from foetuses of thirty days of age were not collected; only amniotic fluid was collected at this age. Because of inadequate samples, the estimated unit concentration of antibody in the amniotic fluid at thirty days depend on a single test i.e. the final test could not be confirmed twice as described in the method of testing. Results obtained from guinea-pigs immunized against tetanus closely agree with those immunized against diphtheria. The C.Q. values for foetal sera ranged from 0.109 at forty-seven days to 2.11 at full term. The C.Q. values for foetal sera

Transfer of antibody (Tetanus antitoxin) from their

Table

Ref	No.	Preg. (days)	Mat. ser. (i.u./ml)	Foet. ser. (i.u./ml.)
378	1L	30	45	-
	2L			-
	3L			-
188	1R	47	165	24
	2R			18
	3R			29
391	1R	50	75	18
	1L			20
	2L			18.5
187	1L	56	195	100
	2L			115
	3L			110
186	1R	60	165	85
	2R			95
	3R			120
	1L			90
177	1	Term	1.2	2.2
	2			2.2
	3			1.5
	4			1.5

the circulation of actively immune guinea-pigs to foetuses

2.

C.Q.	Amn. fluid (i.u./ml.)	C.Q.
	<C.05	<C.CC11
	caC.01	caC.CC022
	caC.C15	caC.CC033
C.145	C.18	C.CC11C
C.109	C.22	C.CC133
C.176	C.24	C.CC152
C.24C	C.09	C.CC12
C.267	C.11	C.CC14
C.246	C.1C	C.CC13
C.512	C.4C	C.CC2C
C.59C	C.38	C.CC19
C.565	C.45	C.CC23
C.515	C.25	C.CC15
C.575	C.35	C.CC21
C.730	C.31	C.CC18
C.545	C.20	C.CC12
1.84	-	
1.84	-	
1.25	-	
1.25	-	

Table 2

Ref. No.		Preg. (days)	Mat. ser. (i.u./ml.)	Foet. ser. (i.u./ml.)
374	1	Term	1.0	1.6
	2			1.65
388	1	Term	2.6	4.3
	2			4.5
	3			5.5

(Continued)

C.Q.	Amn. fluid (i.u./ml.)	C.Q.
1.6	-	
1.65	-	
1.66	-	
1.73	-	
2.11	-	

- = no sample

increased from 0.515 at sixty days to four times as much at full term.

Figure 13 shows the relation between the C.Q. of diphtheria and tetanus antitoxins in the foetal sera (measured in a logarithmic scale) and age after mating. There is a graduál increase in the C.Q. from 39 days after mating to 60 days. A sudden increase in the concentration of both antitoxins occurred between 60 days and full term.

The amniotic fluid C.Q. values for tetanus antitoxin increased tenfold between thirty and fifty-six days of gestation.

Fig. 14 shows the relation between the C.Q. of diphtheria and tetanus antitoxin in the amniotic fluid and age after mating. The C.Q. value for either antitoxin seems to remain constant from about 54 days of gestation to 60 days (the latest age tested). The two encircled values are those thought to be contaminated with blood.

(2) Transfer of heterologous antitoxins from the maternal to the foetal circulation and amniotic fluid:

Pregnant guinea-pigs that were immunized against either diphtheria or tetanus were passively immunized by intracardiac injections of various heterologous antitoxins twenty-four hours before killing. Diphtheria and tetanus antitoxins obtained from rabbit, man and horse were administered at various periods of gestation. Horse

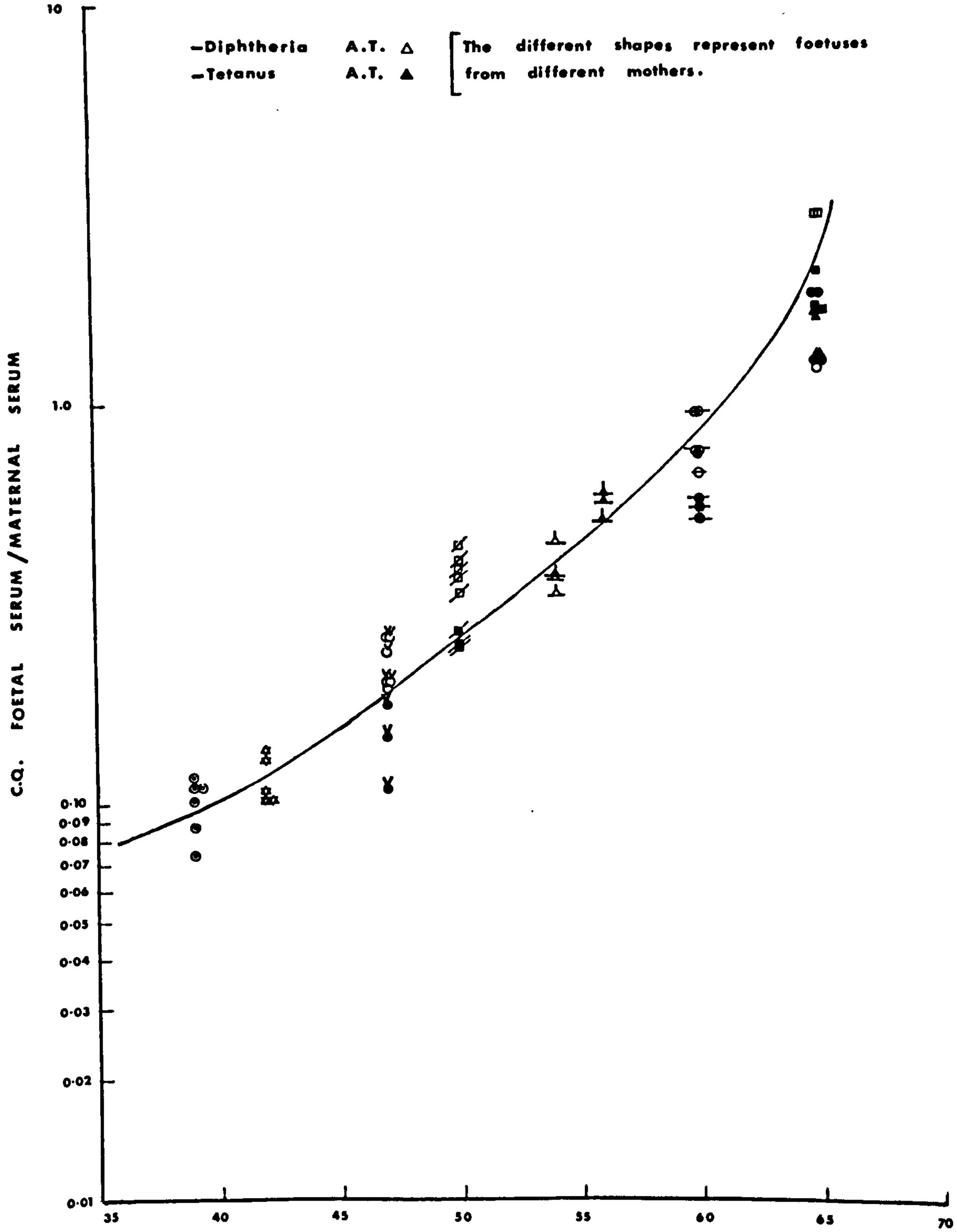


Fig. (13) Relation between concentration quotients of antitoxins in the foetal sera of actively immunized guinea-pigs and age after mating.

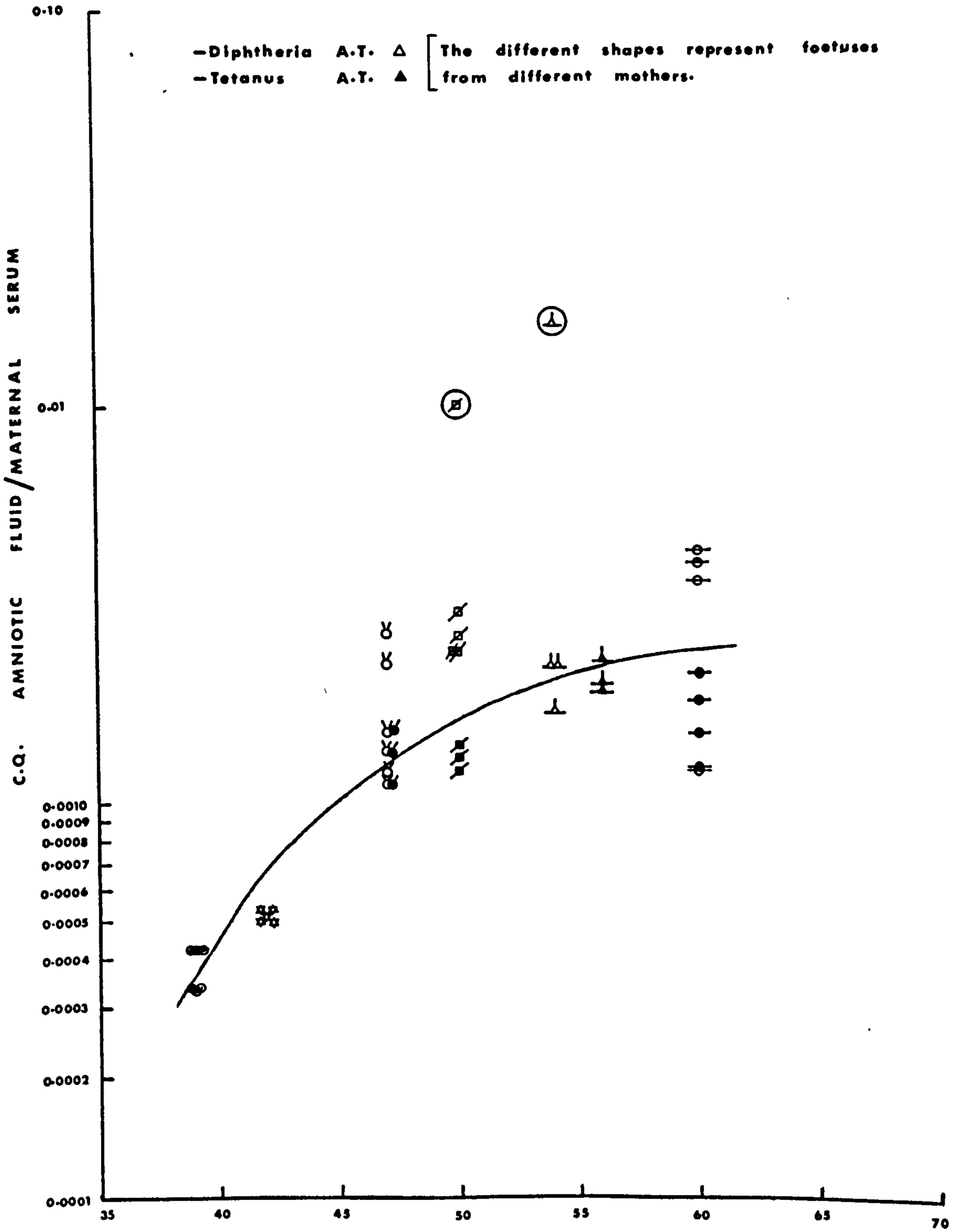


Fig. (14) Relation between concentration quotients of antitoxins in the amniotic fluids of actively immunized guinea-pigs and age after mating.

tetanus antitoxin was either crude or refined. In one experiment (guinea-pig 342) the pregnant guinea-pig was non-immune; it received an injection of a mixture containing human tetanus and guinea-pig diphtheria antitoxin. Maternal and foetal sera and amniotic fluids were collected at killing and their values were estimated as i.u. per ml.; the C.Q. values for foetal sera and amniotic fluids were calculated. Results are shown in table 3. Because most of the samples were small in quantity and low in value, some of the tests were not carried to finality; values were expressed as less than the lowest value tested. Some of the samples were tested only once i.e., the final test was not confirmed twice as described in the method. In such case the values were expressed as circa (ca). In two foetuses (guinea-pig 369 1R and 3R) no amniotic fluid was available for any testing, but three amniotic fluid samples from litter mates (guinea-pig 369 2R, 4R and 1L) were tested.

Results showed that the C.Q. values for foetal sera were much lower than those obtained from foetuses of actively immune mothers. However, a direct comparison between these two experiments was not possible since the foetuses of actively immune mothers were exposed to the immune serum for a much longer time than those of the present experiment.

Transfer of heterologous antitoxin from the maternal to

Table

Ref No.	Preg. (days)	Antitoxin in the maternal serum (i.u./ml.) Tetanus	Diphtheria
368 1R	47	1.35 (Rab.)	-
2R			
3R			
1L			
2L			
3L			
391 1R	50	-	8 (Rab.)
1L			
2L			
363 1R	50	3 (Hum.)	-
2R			
3R			
4R			
1L			
342 1R	53	5 (Hum.)	6 (G.-pig)
1L			
2L			
3L			
4L			
5L			

the foetal circulation and amniotic fluid

3.

Antitoxin in the foetal serum

(i.u./ml.)		(C.Q.)	
Tet:	Diph.	Tet.	Diph.
ca0.01	-	ca0.0073	-
<0.01	-	<0.0073	-
<0.02	-	<0.0149	-
ca0.02	-	ca0.0149	-
ca0.02	-	ca0.0149	-
ca0.02	-	ca0.0149	-
-	0.1	-	0.0125
-	0.1	-	0.0125
-	0.1	-	0.0125
-	0.1	-	0.0125
0.110	-	0.036	-
0.135	-	0.045	-
0.135	-	0.045	-
0.120	-	0.040	-
0.135	-	0.045	-
<0.010 >0.005	0.067	<0.0020	0.011
ca0.018	0.067	ca0.0036	0.011
<0.020 >0.01	0.067	<0.0040	0.011
<0.050 >0.01	0.083	<0.010	0.013
ca0.020	0.083	ca0.0040	0.013
ca0.015	0.067	ca0.0030	0.011

the foetal circulation and amniotic fluid

3.

Antitoxin in the foetal serum (i.u./ml.)		Antitoxin in the foetal serum (C.Q.)	
Tet:	Diph.	Tet.	Diph.
ca0.01	-	ca0.0073	-
<0.01	-	<0.0073	-
<0.02	-	<0.0149	-
ca0.02	-	ca0.0149	-
ca0.02	-	ca0.0149	-
ca0.02	-	ca0.0149	-
-	0.1	-	0.0125
-	0.1	-	0.0125
-	0.1	-	0.0125
-	0.1	-	0.0125
0.110	-	0.036	-
0.135	-	0.045	-
0.135	-	0.045	-
0.120	-	0.040	-
0.135	-	0.045	-
<0.010 >0.005	0.067	<0.0020	0.011
ca0.018	0.067	ca0.0036	0.011
<0.020 >0.01	0.067	<0.0040	0.011
<0.050 >0.01	0.083	<0.010	0.013
ca0.020	0.083	ca0.0040	0.013
ca0.015	0.067	ca0.0030	0.011

Antitoxin in the amniotic fluid (i.u./ml.)		Antitoxin in the amniotic fluid C.Q.	
Tet.	Diph.	Tet.	Diph.
<0.002	-	<0.0014	-
<0.002	-	<0.0014	-
--	-	.	-
<0.005	-	<0.0037	-
<0.002	-	<0.0014	-
<0.002	-	<0.0014	-
-	ca0.001	-	ca0.00012
-	<0.0005	-	<0.00006
-	<0.0005	-	<0.00006
-	<0.0005	-	<0.00006
<0.005	-	<0.0016	-
<0.005	-	<0.0016	-
<0.005	-	<0.0016	-
ca0.005	-	ca0.0016	-
<0.005	-	<0.0016	-
<0.002	<0.0005	<0.0004	<0.00008
<0.002	<0.0005	<0.0004	<0.00008
<0.002	<0.0005	<0.0004	<0.00008
<0.002	<0.001	<0.0004	<0.00016
<0.002	<0.001	<0.0004	<0.00016
<0.002	<0.0005	<0.0004	<0.00008

Table 3

Antitoxin in the maternal serum (i.u./ml.)

Ref No.	Preg. (days)	Tetanus	Diphtheria
168 1R	54	C.88 (horse, crude)	-
2R			
1L			
2L			
369 1R	60	4 (horse, refined)	-
2R			
3R			
4R			
1L			

(Continued)

Antitoxin in the foetal serum (i.u./ml.)		Antitoxin in the foetal serum (C.Q.)	
Tet.	Diph.	Tet.	Diph.
< C.CC2	-	< C.CC22	-
< C.CC2	-	< C.CC22	-
< C.CC2	-	< C.CC22	-
< C.CC2	-	< C.CC22	-
caC.C2C	-	caC.CC5	-
C.C19	-	C.CC47	-
C.C18	-	C.CC45	-
C.C165	-	C.CC41	-
C.C19	-	C.CC47	-

Antitoxin in the amniotic fluid (i.u./ml.)		Antitoxin in the amniotic fluid (C.Q.)	
Tet.	Diph.	Tet.	Diph.
< C.CC5	-	< C.CC57	-
< C.CC5	-	< C.CC57	-
< C.CC2	-	< C.CC22	-
< C.CC2	-	< C.CC22	-
--	-		-
< C.CC2	-	< C.CCC5	-
--	-		-
< C.CC6	-	< C.CC15	-
< C.CC2	-	< C.CCC5	-

samples marked ca were tested only once.

-- = no sample

A C.Q. of about 0.01 of rabbit antitoxin in the foetal circulation was obtained at forty-seven and fifty days of gestation. Human antitoxin entered the foetal circulation at fifty days gestation with a higher rate:; the C.Q. value was about 0.04. However, at fifty-three days gestation human antitoxin entered the foetal circulation at a much lower rate; the C.Q. values were about 0.004. I have not yet thought of any explanation for this.

In experiment 168 at fifty-four days of gestation in which horse crude antitoxin was injected into the maternal circulation, all serum and half the amniotic fluid values were lower than the limit of testing for tetanus i.e. less than 0.002 i.u. per ml. The unit value for antitoxin circulating in the mother was also low, 0. 88i.u. per ml. It was unfortunate that the horse antitoxin available contained preservative so that it had to be diluted five times with saline before injection into the maternal circulation.

Refined tetanus antitoxin prepared in the horse was injected into the maternal circulation at sixty days of gestation. The C.Q. values for foetal sera ranged from 0.0041 to 0.0047.

In experiment 342 a direct comparison between the entry of heterologous (human) antiserum to that of homologous antiserum was possible in the same animal.

In this experiment the transfer of homologous antitoxin to the foetal circulation at fifty-three days of gestation was slightly over twice as great as that for the heterologous antitoxin.

The entry of heterologous antitoxin from the maternal circulation into the amniotic fluid was so low that almost all the samples tested were expressed as less than the lowest possible testing value.

(3) Transfer of antibody from the uterine cavity to the foetus:

Various mixtures of antitoxins were injected directly into the uterine cavity of non-immune pregnant guinea-pigs at various periods of gestation. The foetuses were exposed to the heterologous and homologous serum simultaneously. The injection was made into only one uterine horn; the other was left as control. In one experiment (guinea-pig 295) the same mixture was injected into both uterine horns. The amount injected was standardized as 0.5 ml. per foetus in all cases; it was rather difficult to inject greater volumes. Samples of maternal and foetal sera and amniotic fluids were collected from twenty to twenty-four hours after administration of antitoxins. Maternal samples were not collected before operations since in earlier experiments maternal samples showed that neither diphtheria nor tetanus antitoxins were normally present

in the circulation. Clostridium welchii α-antitoxin was also found to be absent in normal guinea-pig serum. Results of transfer of antibodies from the uterine cavity to the foetal circulation and amniotic fluid are recorded in table 4. Samples that were either not enough for testing (guinea-pig 405 3R, amniotic fluid and 332 2L, serum) were tested as far as material permitted. In all cases the transfer of antibodies to the foetal circulation seemed to be selective in nature in that the heterologous antitoxins were transferred less readily than the homologous. The C.Q. values for entry of homologous antisera to the foetal circulation were always higher than those for heterologous in the same animal at all gestation periods tested. These results indicated that the rate of transfer of homologous antitoxin to the foetal circulation was higher than that of heterologous antitoxin.

The C.Q. values for entry of guinea-pig antitoxin was twice as great as that for rabbit antitoxin at forty-one days of gestation (guinea-pig 119), nearly twice as great at forty-five days (guinea-pig 338) and three times as great at forty-six and fifty-six days (guinea-pigs 348 and 332).

The transfer of human antitoxin to the foetal circulation was five times less than that of guinea-pig antitoxin at forty-seven and fifty-six days of gestation (guinea-pigs 407 and 343). In experiment 325 at

Transfer of antibody injected into the uterine cavity of

and amniotic

Table

Ref. No.		Preg. (days)	Mixture injected (i.u./ml.) Tet.	Diph.	Cl.welchii
119	1R	41	10 (R)	10 (G)	-
	2R				
	1L(Con)				
295	1R	44	360 (H.R.)	135 (G)	-
	1L				
292	1L	45	75 (H.C.)	36 (G)	132 (C)
	2L				
	3L				
338	1R(Con)	45	50 (G)	55 (R)	-
	1L				
	2L				
348	1L	46	55 (G)	75 (R)	-
	2L				
	3L				
407	1R(Con)	47	75 (Hu.)	75 (G)	-
	1L				
	2L				

non-immune pregnant guinea-pigs to the foetal circulation fluid.

4.

Foetal serum (i.u./ml.)			C.Q.		
Tet.	Diph.	Cl.welchii	Tet.	Diph.	Cl.welchii
0.08	0.25	-	0.005	0.025	-
0.08	0.25	-	0.005	0.025	-
<0.01	<0.001	-	<0.0006	<0.0001	-
<0.2	3.0	-	<0.0006	0.022	-
0.18	3.3	-	0.0005	0.024	-
<0.01	0.36	0.10	<0.00013	0.010	0.00075
<0.01	0.42	<0.01	<0.00013	0.011	<0.000075
<0.01	0.17	0.14	<0.00013	0.018	0.00106
<0.01	<0.01	-	<0.0002	<0.00018	-
0.36	0.12	-	0.0072	0.0021	-
0.165	<0.12	-	0.0033	<0.0021	-
1.3	0.6	-	0.023	0.008	-
1.2	0.55	-	0.022	0.007	-
1.1	0.5	-	0.020	0.007	-
<0.005	<0.0005	-	<0.00006	<0.000006	-
0.52	2.9	-	0.007	0.037	-
0.50	2.7	-	0.007	0.036	-

Amniotic fluid (i.u./ml.)			C.Q.		
Tet.	Diph.	Cl.welchii	Tet.	Diph.	Cl. welchii
<0.002	<0.001	-	<0.0001	<0.0001	-
<0.002	<0.001	-	<0.0001	<0.0001	-
<0.002	<0.001	-	<0.0001	<0.0001	-
ca0.010	0.0020	-	ca0.000029	0.000014	-
ca0.011	0.0025	-	ca0.000030	0.000018	-
<0.1	<0.001	0.005	<0.0001	<0.00002	0.00003
<0.001	<0.005	<0.002	<0.00001	<0.0001	<0.000015
--	--	--			
<0.002	<0.0005	-	<0.0004	<0.00009	-
<0.002	<0.001	-	<0.0004	<0.00001	-
<0.002	ca0.001	-	<0.0004	ca0.00001	-
<0.005	0.008	-	<0.0009	0.0001	-
<0.005	0.005	-	<0.0009	0.00006	-
ca0.05*	0.05	-	0.009	0.0006	-
<0.002	<0.0005	-	<0.000026	<0.000006	-
<0.002	ca0.001	-	<0.000026	ca0.000013	-
<0.002	ca0.001	-	<0.000026	ca0.000013	-

(Con) = control (G) = guinea-pig (Hu) = human

(H.R.) = Horse, refined (H.C.) = Horse, crude

(C) = cow

* may be contaminated with blood

-- = no sample

Table 4

Ref. No.		Preg. (days)	Mixture injected (i.u./ml.) Tet.	Diph.	Cl.welchii
296	1R(Con)	47	4C(R)	52(G)	128(C)
	1L				
	2L				
	3L				
325	1R(Con)	47	17(Hu.)	16.5(G)	125(C)
	1L				
	2L				
	3L				
	4L				
405	1R	52	16C(H.C.)	65(G)	-
	2R				
	3R				
	1L(Con)				
403	1R	53	17C(H.C.)	70(G)	-
	2R				
	3R				
332	1L	56	165(R)	55(G)	-
	2L				
	3L				
343	1R	56	7C(Hu.)	5C(G)	-

(Continued)

Foetal serum (i.u./ml.)			C.Q.		
Tet.	Diph.	Cl.welchii	Tet.	Diph.	Cl.welchii
<0.025	ca0.005	-	<0.00062	ca0.00009	-
<0.025	ca0.06	-	<0.00062	ca0.00115	-
<0.020	0.0165	<0.04	<0.0005	0.00032	<0.00031
<0.025	0.014	<0.01	<0.00062	0.00027	<0.00007
<0.025	-	<0.16	<0.0014	-	<0.009
<0.02	0.02	0.04	<0.0011	0.0012	0.0003
<0.01	0.037	<0.01	<0.0006	0.0021	<0.00008
<0.01	0.038	<0.02	<0.0006	0.0021	<0.0001
<0.01	0.037	<0.02	<0.0006	0.0021	<0.0001
ca0.02	2.2	-	ca0.00012	0.034	-
ca0.03	2.7	-	ca0.00018	0.041	-
ca0.018	1.8	-	ca0.00005	0.028	-
(i.s.)	<0.01	-	-	<0.0001	-
0.022	2.0	-	0.00012	0.028	-
0.02	1.2	-	0.00011	0.017	-
<0.01	0.3	-	<0.00007	0.004	-
1.35	1.35	-	0.008	0.024	-
(i.s.)	ca1.0	-		0.018	-
0.65	0.55	-	0.003	0.010	-
0.08	0.24	-	0.0011	0.005	-

(i.s.) = insufficient sample

-- = no sample

Amniotic fluid (i.u./ml.)			C.Q.		
Tet.	Diph.	Cl.welchii	Tet.	Diph.	Cl.welchii
<0.002	0.0017	-	<0.0005	<0.00003	-
-	0.028	0.059	-	0.00053	0.0004
<0.002	-	-	<0.0005	-	-
<0.025	<0.010	<0.002	<0.0006	<0.0001	<0.0001
<0.001	<0.001	<0.002	<0.00005	<0.00006	<0.00001
<0.001	<0.001	<0.002	<0.00005	<0.00006	<0.00001
<0.001	<0.001	<0.002	<0.00005	<0.00006	<0.00001
<0.001	<0.001	<0.002	<0.00005	<0.00006	<0.00001
<0.001	<0.001	<0.002	<0.00005	<0.00006	<0.00001
<0.005	0.0025	-	<0.00003	0.00003	-
<0.005	0.005	-	<0.00003	0.00006	-
(i.s.)	<0.01	-	-	<0.00015	-
<0.002	<0.001	-	<0.00001	<0.00001	-
<0.002	<0.001	-	<0.00001	<0.00001	-
ca0.1*	ca0.24*	-	ca0.0005	ca0.003	-
--	--	-			-
--	--	-			-
--	--	-			-
--	--	-			-
<0.002	0.0016	-	<0.00029	0.00003	-

forty-seven days, although the transfer of homologous antitoxin was low it was still higher than that of the human antitoxin in two foetuses.

Horse antitoxin was transferred to the foetal circulation at a C.Q. at least 100 times less than that of guinea-pig antitoxin at forty-five days of gestation (guinea-pig 292), about 200-300 times less at fifty-two days (guinea-pig 405) and about 230 times less at fifty-three days (guinea-pig403). The transfer of refined horse antitoxin to the foetal circulation was at least fifty times less than that of homologous antitoxin at forty-four days of gestation (guinea-pig 295).

Cow antitoxin was transferred to the foetal circulation in concentrations 13 to 16 times less than guinea-pig antitoxin at forty-five days of gestation (guinea-pig 292). At forty-seven days of gestation guinea-pig antitoxin was transferred to the foetal circulation at concentrations more than 21 times greater than cow antitoxin (guinea-pig 325).

The arrangement of foetuses in the uterus appeared to influence the C.Q. values for transfer of antitoxin into the foetal circulation. This was clearly shown in guinea-pig 348, where the serum of foetus 1L had a higher C.Q. for both homologous and heterologous transfer of antibodies than that of foetuses 2L and 3L. This was expected since 1L was nearest to the site of injection

and the guinea-pig foetus fills the uterus much more than the rabbit foetus does. Similar results were observed in experiments 332 and 403. In experiment 405 the arrangement of foetuses seemed to affect the entry of antibodies into the foetal circulation in a different manner. There seemed to be a pool of the mixture injected near foetus 2R, for higher C.Q. values for this foetal serum were observed.

The transfer of antitoxins to the amniotic fluid was much lower than that to the foetal circulation. In almost all the samples the amniotic fluid antitoxin concentration values were below the level of testing. In only one sample (guinea-pig 348 3L) the value was high, this might be due to contamination with blood.

It has been observed in these experiments that antitoxins injected into the uterine cavity passed freely into the maternal circulation at all stages of gestation tested (table 5). This passage appears to be non-selective in that heterologous antitoxins passed at the same C.Q. as homologous antitoxins and the C.Q. was independent of the stage of gestation.

Transfer of antitoxins from the uterine

Table

Ref.No.	Preg. (days)	Whether one or both uteri injected
119	41	1
295	44	2
292	45	1
338	45	1
348	46	1
407	47	1
296	47	1
325	47	1
405	52	1
403	53	1
332	56	1
343	56	1

cavity into the maternal circulation.

5.

Mixture injected (i.u./ml.)			Maternal serum (i.u./ml.)					
Tet.	Diph.	Cl. welchii α-antitoxin.	Tet.	Diph.	α-antitoxin	Tet.	C.Q. Diph.	α-antitoxin
16 (R)	10 (G)	-	-	< 0.001	-	-	< 0.0001	-
360 (H.R.)	135 (G)	-	0.22	< 0.005	-	0.0006	< 0.00003	-
75 (H.C.)	30 (G)	132 (C)	0.24	0.17	0.45	0.0032	0.0047	0.0034
50 (G)	55 (R)	-	0.15	0.22	-	0.0030	0.004	-
55 (G)	75 (R)	-	< 0.1	< 0.001	-	< 0.0018	< 0.00001	-
75 (Hu.)	75 (G)	-	ca 0.18	0.15	-	ca 0.0024	0.0020	-
40 (R)	52 (G)	128 (C)	0.33	0.43	0.60	0.0082	0.0083	0.0046
17 (Hu.)	16.5 (G)	125 (C)	0.25	0.30	ca 0.60	0.015	0.018	ca 0.0048
160 (H.C.)	65 (G)	-	0.36	0.14	-	0.0022	0.0023	-
170 (H.C.)	70 (G)	-	0.33	0.10	-	0.0019	0.0014	-
165 (R)	55 (G)	-	0.45	0.16	-	0.0027	0.0029	-
70 (Hu.)	50 (G)	-	0.065	0.04	-	0.0009	0.0008	-

(4) Transfer of antibody from the maternal circulation and from the uterine cavity to the stomach contents of foetal guinea-pigs:

Stomach content samples were obtained from foetal guinea-pigs of actively immunized mothers or of non-immune mothers injected with a mixture of homologous and heterologous antitoxin into the uterine cavity or the circulation at various stages of gestation. Results are presented in table 6. Because the stomach samples were so low in value and so small in quantities only limited tests were possible. Those samples that were not confirmed twice were recorded as ca.; other samples were recorded as less than the lowest value tested. Unlike the rabbit, the guinea-pig foetus showed no evidence of concentration of antitoxins in its stomach.

(5) Transfer of antibody from the stomach to the circulation of newborn guinea-pigs:

Newborn guinea-pigs of two to twenty-two hours of age were fed through a polythene stomach tube with homologous and various heterologous antitoxins prepared in rabbit, man, dog and horse. The number of units given varied from 10 to 330. In some cases the antiserum given was divided into two equal parts and the half volumes were given with an interval of 80 minutes. Blood samples were collected twenty-four hours after feeding and their values were determined as far as material permitted.

Transfer of antitoxin from the maternal circulation and guinea-pig

Table

Ref.No.	Prec.(days)	Maternal serum (i.u./ml.) Tet.	Diph.
295 1R	44	-	-
1L			
292 1L	45	-	-
2L			
348 1L	46	-	-
2L			
325 1R (Con)	47	-	-
1L			
2L			
3L			
4L			
407 1R (Con)	47	-	-
1L			
391 1R	50	75 (G)	8 (R)
1L			

from the uterine cavity to the stomach contents of foetuses.

6.

Antitoxin mixture injected into the uterine cavity (i.u./ml.)		Artitoxin present in the foetal stomach contents (i.u./ml.)			
Tet.	Diph.	Tet.	Diph.	Tet.	Diph.
360 (H.R.)	135 (G)	(i.s.)	<0.005	-	<0.00003
		(i.s.)	<0.005	-	<0.00003
75 (H.C.)	30 (G)	<0.02	<0.01	<0.00026	<0.00027
		<0.01	<0.01	<0.00013	<0.00027
55 (G)	75 (R)	(i.s.)	<0.006	-	<0.00008
		(i.s.)	<0.006	-	<0.00008
17 (Hu.)	16.5 (G)	<0.05	<0.01	<0.003	<0.0006
		<0.02	<0.01	<0.0012	<0.0006
		<0.03	<0.01	<0.0017	<0.0006
		<0.03	<0.01	<0.0017	<0.0006
75 (Hu.)	75 (G)	<0.05	<0.01	<0.003	<0.0006
		(i.s.)	<0.0005	-	<0.000006
		(i.s.)	<0.001	-	<0.00001
-	-	(i.s.)	<0.01	-	<0.0012
		(i.s.)	ca0.002	-	ca0.00025

Table 6.

Ref.No.		Pres.(days)	Maternal serum (i.u./ml.) Tet.	Diph.
403	1R	53	-	-
	2R			
342	1R	53	5 (Hu.)	6 (G)
	1L			
	2L			
	3L			
	4L			
332	1L	56	-	-
	2L			

(Continued)

Antitoxin mixture injected into the uterine cavity (i.u./ml.)		Antitoxin present in the foetal stomach contents (i.u./ml.)			
				C.Q.	
Tet.	Diph.	Tet.	Diph.	Tet.	Diph.
170 (H.C.)	70 (G)	<0.01	<0.001	<0.00005	<0.00001
		<0.01	<0.001	<0.00005	<0.00001
-	-	<0.02	<0.001	<0.004	<0.00016
		<0.02	<0.002	<0.004	<0.00033
		<0.02	<0.002	<0.004	<0.00033
		<0.02	<0.002	<0.004	<0.00033
		<0.02	<0.002	<0.004	<0.00033
165 (R)	55 (G)	<0.02	<0.1	<0.0001	<0.001
		<0.02	<0.1	<0.0001	<0.001

The serum values were so low that the majority of samples had to be recorded as less than the lowest value tested.

Table 7 shows the relation between the amount given and that present in the circulation i.e. the C.Q. values. In two cases fed with antitoxins at two hours after birth the C.Q. values of the newborn sera were 0.0001 and 0.00007 for guinea-pig and rabbit antitoxin respectively. The number of units given were 110 in case of guinea-pig antitoxin and 135 in case of rabbit antitoxin. However, when nearly three times as many units of rabbit and guinea-pig antitoxin were given at twelve hours of age, the C.Q. values were <0.000003 and <0.000006 respectively.

When forty units of dog tetanus antitoxin was given at twenty hours of age no antitoxin (i.e., <0.005 units per ml. serum) was detected in the newborn serum. When 90 units of horse tetanus antitoxin was given at twenty hours of age, no antitoxin (i.e., <0.002 units per ml.) was detected. The corresponding C.Q. values for newborn guinea-pigs fed at twenty hours of age with dog and horse tetanus antitoxin were <0.000125 and <0.000022 respectively.

Transfer of antibody from the stomach to

Table

Ref No.		Weight (gm.)	Age (hrs.)	Total units fed (i.u./ml.)
331	1	86.1	2	135
	2	102.0	2	135
	3	84.0	2	110
254	1	70.0	12	10
	2	60.7	12	15
	3	74.8	12	20
	4	78.1	12	none
292	1	78.5	12	330
	2	78.0	12	300
328	1	78.0	20	20
	2	72.5	20	27.5
312	1	78.0	20	200
	2	74.5	20	200
	3	76.5	20	none
85	1	62.5	20	40
	2	57.0	20	280
	3	83.0	20	90
327	1	85.0	22	50
	2	91.0	22	50

the circulation of newborn guinea-pigs.

7.

Type	Species of origin	Foetal ser. (i.u./ml.)	C.C.
Diph.	Rabbit	0.01	0.000073
"	"	< 0.02	< 0.000146
"	Guinea-pig	0.01	0.0001
Tet.	Guinea-pig	< 0.002	< 0.0002
"	" "	< 0.002	< 0.00013
"	" "	< 0.002	< 0.0001
-	-	< 0.002	non-toxic
Tet.	Guinea-pig	< 0.002	< 0.000006
Diph.	Rabbit	< 0.001	< 0.000003
Tet.	Rabbit	< 0.002	< 0.0001
"	"	< 0.002	< 0.00006
Tet.	Guinea-pig	< 0.01	< 0.00005
"	" "	< 0.02	< 0.0001
-	-	< 0.002	non-toxic
Tet.	Dog	< 0.005	< 0.000125
"	Guinea-pig	< 0.020	< 0.00006
"	Horse	< 0.002	< 0.000022
Diph.	Guinea-pig	< 0.01	< 0.002
"	"	< 0.001	< 0.00002

(6) Transfer of natural and pepsin-refined antitoxins prepared in the guinea-pig from the uterine cavity to the foetus:

Four guinea-pigs at the forty-fifth day of gestation received an intra-uterine injection of a mixture of pepsin-refined guinea-pig tetanus antitoxin and whole (unrefined) guinea-pig diphtheria antitoxin; one horn was left as control. The amount injected was 0.5 ml. per foetus in each case. Maternal and foetal serum samples, amniotic fluid and stomach contents were collected twenty-four hours later.

Table 8 shows the concentration of diphtheria and tetanus antitoxin present in the foetal serum expressed as i.u. per ml. and as C.Q. Refined tetanus antitoxin was transferred to the foetal circulation much less readily than crude diphtheria antitoxin. Because of insufficient amounts of foetal sera the real number of units of tetanus antitoxin per ml. were difficult to estimate, however, it was possible to test as low as 0.01 unit in certain cases. The C.Q. values for tetanus antitoxin in the foetal sera did not exceed those of the controls; whilst those for diphtheria antitoxin reached a value as high as 0.075.

Transfer of diphtheria (natural) and tetanus (refined) the uterine cavity on the 45th day of gestation to the

Table

Ref. No.		diphtheria (natural) antitoxin (i.u./ml.) serum injected	foetal serum
305	1R (Con)	37	0.067
	1L		1.3
	2L		1.15
308	1R (Con)	30	<0.01
	1L		0.95
337	1R	41	3.1
	2R		1.51
	3R		1.7
	1L (Con)		<0.01
	2L (Con)		0.012
339	1R	36	0.019
	2R		0.018
	3R		0.025
	4R		0.028
	1L (Con)		<0.010

antitoxin prepared in the guinea-pig and injected into circulation of the foetal guinea-pig after 24 h. exposure &.

Tetanus (refined) antitoxin (i.u./ml.)		C.Q.	
serum injected	foetal serum	Diph.	Tet.
23	< 0.01	0.0018	< 0.00043
	< 0.01	0.035	< 0.00043
	< 0.01	0.031	< 0.00043
24	< 0.01	< 0.00034	< 0.00041
	< 0.01	0.031	< 0.00041
21	< 0.02	0.075	< 0.0009
	< 0.012	0.036	< 0.0005
	< 0.02	0.041	< 0.0009
	< 0.02	< 0.0002	< 0.0009
	< 0.02	0.0003	< 0.0009
22.5	< 0.02	0.00052	< 0.0008
	< 0.02	0.0005	< 0.0008
	< 0.02	0.0007	< 0.0008
	< 0.02	0.0007	< 0.0008
	< 0.02	< 0.0002	< 0.0008

Table 9 shows the concentration of diphtheria and tetanus antitoxin in the amniotic fluid and stomach content samples. No stomach content samples were collected from guinea-pig 308. Foetus 2L of guinea-pig 337 had no amniotic fluid. The stomach content samples were so low in value that the amounts available were insufficient to carry the tests to finality. All stomach content samples were expressed as less than the lowest value tested. The C.Q. for diphtheria antitoxin in the stomach contents of foetuses of the injected horn ranged from <0.0003 to <0.019; that for tetanus from <0.003 to <0.14. The C.Q. for diphtheria antitoxin in the amniotic fluid of foetuses of the injected horn ranged from <0.00002 to <0.00005; that for tetanus from <0.00004 to <0.00008.

Table 10 shows the transfer of diphtheria and tetanus antitoxin from the uterine cavity into the maternal circulation. Values were expressed as i.u. per ml. and as C.Q. Diphtheria and tetanus antitoxin passed at an almost equal rates; the C.Q. values for diphtheria and tetanus antitoxin in the maternal circulation were about equal.

Transfer of diphtheria (natural) and tetanus (refined) the uterine cavity on the 45th day of gestation to the guinea-pig after

Ref. No.		serum injected (i.u./ml.) Diphtheria (natural) A.T	Tetanus (refined) A.T
305	1R (Con)	37	23
	1L		
	2L		
308	1R (Con)	30	24
	1L		
337	1R	41	21
	2R		
	3R		
	1L (Con)		
	2L (Con)		
339	1R	36	22.5
	2R		
	3R		
	4R		
	1L (Con)		

artitoxin prepared in the guinea-pig and injected into amniotic fluid and stomach content of the foetal 24 hr. exposure

Table 9.

amniotic fluid				stomach content			
i.u./ml.		C.Q.		i.u./ml.		C.Q.	
diph.	tet.	diph.	tet.	diph.	tet.	diph.	tet.
< C.CC1	< C.CC1	< 0.CCCC2	< C.CCCC4	< 0.C4	< 0.04	< C.CC10	< C.CC17
< C.CC1	< C.CC1	< C.CCCC2	< C.CCCC4	< 0.C7	< 0.07	< C.CC18	< 0.CC3C
< C.CC12	< C.CC2	< 0.CCCC3	< 0.CCCC8	< C.C74	< 0.C74	< C.CC20	< C.CC32
< C.02	< C.C2	< C.CCC6	< C.CCC8	--	--		
< C.CC1	< 0.CC2	< C.CCCC3	< C.CCCC8	--	--		
< C.CC1	< 0.CC1	< 0.CCCC25	< C.CCCC4	C.C3	< 0.77	< C.C19	< 0.C36
< C.CC1	< C.CC1	< C.CCCC25	< C.CCCC4	--	--		
< C.CC1	< C.CC1	< C.CCCC25	< C.CCCC4	< C.C16	< 0.16	< C.CC4	< C.CC7
< C.CC1	< C.CC1	< C.CCCC25	< C.CCCC4	< C.C18	< 0.18	< C.CC45	< C.CC8
< C.CC1	< C.CC1	< C.CCCC25	< C.CCCC4	< C.C13	< C.13	< C.CC3	< C.CC6
--	--						
< C.CC1	< C.CC1	< C.CCCC3	< C.CCCC4	< C.C5	< C.5	< C.CC14	< C.C22
< C.CC1	< C.CC1	< C.CCCC3	< C.CCCC4	< C.C33	< 0.33	< C.CCC9	< C.C14
< C.CC17	< C.CC1	< C.CCCC5	< C.CCCC4	< C.C11	< C.11	< C.CCC3	< C.CC4
< C.CC1	< C.CC1	< C.CCCC3	< C.CCCC4	< C.C18	< C.18	< C.CCC5	< C.CC8
< C.CC1	< C.CC1	< C.CCCC3	< C.CCCC4	< C.C25	< 0.25	< C.CCC7	< C.C11

-- = no sample

Transfer of diphtheria (natural) and tetanus (refined) the uterine cavity on the 45th day of gestation to

Ref. No.	mixture injected (i.u./ml.) Tet. (refined)	Diph. (natural)
305	23	37
308	24	30
337	21	41
339	22.5	36

antitoxin prepared in the guinea-pig and injected into the maternal circulation.

Table 1C

Maternal serum (i.u./ml.)		C.Q.	
Tet.	Diph.	Tet.	Diph.
0.0165	0.038	0.0007	0.0010
0.011	0.016	0.0004	0.0005
0.036	0.15	0.0017	0.003
0.09	0.25	0.004	0.007

DISCUSSION

It is evident from our data that the guinea-pig is born fully equipped with antibodies that were present in the maternal circulation during pregnancy. Tables 1 and 2 show that the young of guinea-pig are born with diphtheria or tetanus antitoxins in their sera nearly three times as concentrated as those present in the maternal sera at parturition. The concentration quotient (C.Q., defined by Batty et al. (1954) as the ratio of antitoxin concentration of the foetal sample to that of the serum to which it was exposed) increased from 0.073 at 39 days of gestation to 2.95 at term (about 65 days). These results closely agree with those of Barnes (1957). She found that the percentage of foetal to maternal titres of diphtheria antitoxin rose from 0.1 at 35 days to 230 per cent. at term. However, Barnes showed that there was a fall in the percentage of antitoxin concentration in the foetal sera from over 260 per cent. on the 63rd day to 230 per cent. at term. No such fall is observed by the present author. On the contrary, it is found in the present work that a rapid accumulation of antitoxins in the foetal sera occurred between 60 days of gestation and term. If one considers Barnes' results carefully, the fall in the percentage of diphtheria antitoxin in the foetal sera to that of the mother is probably not significant. This fall amounts to 11.5 per cent. which

is near the variation between litters. In the rabbit however, Batty et al. (1954) found that there was a decline in the C.Q. of foetal serum after 26 days post-coitum when the foetuses were exposed to immune rabbit serum for 24 hours.

Figure 13 shows the relation between the C.Q. of diphtheria or tetanus antitoxins of foetal sera and the age of the foetuses in days after mating. A gradual increase in the C.Q. of antitoxins present in the foetal sera is observed as gestation advance accompanied by a sudden accumulation of antitoxins between the 60th day and term. The gradual increase can be explained by the accumulation of antitoxin as gestation advanced i.e., the longer the time of exposure the higher the concentration of antitoxin in the foetal sera. Hartley (1948) found that the time when guinea-pig diphtheria antitoxin is injected into non-immune pregnant guinea-pigs affects the concentration of the antitoxin attained in the young. The longer the interval between injection and parturition the more antitoxin is concentrated in the foetal circulation. However, time alone does not explain the sudden increase in the C.Q. of antitoxins of foetal sera from 0.67 to 2.95 in the case of diphtheria and from 0.515 to 2.11 in the case of tetanus between 60 days of gestation and term. This sudden increase can be explained by an increase in the

permeability of the membranes and by the ability of the foetus to concentrate antitoxins against a concentration gradient. In rabbits, Rodolfo (1934) found that between 22 and 30 days of gestation the ratio of foetal to maternal titres of agglutinins and haemolysins increased as gestation advanced. When he plotted the ratio of foetal to maternal titres against days of gestation a sigmoid curve was obtained. Rodolfo explained his finding by an increase in the permeability of the placenta. In man, globulin in premature infants increased from twenty weeks to full term (Remington and Pickford, 1947). Osborn, Dancis and Rosenberg (1952) found that in man as birth weight increased there was an increase in the permeability of the placenta.

In the guinea-pig, as in the rabbit, the route of transfer of antibodies to the foetus is from the maternal circulation via the uterine cavity, the yolk-sac splanchnopleur and the vitelline circulation (Brambell, Hemmings, Henderson, Parry and Rowland,1949; Barnes, 1957). A comparison between the arrangement of foetal membranes of guinea-pig and rabbit shows the similarity between this arrangement and the route of transfer of antibodies. Rats also exhibit an arrangement of foetal membranes similar to that in guinea-pigs and rabbits. Although rats acquire their passive immunity mainly through the milk, a significant amount of antibodies is

transferred before birth (Halliday, 1955b). The transfer of antibodies before birth in rats is also via the yolk-sac splanchnopleur and vitelline circulation; however, a direct passage of antibodies via the placenta was not excluded (Brambell and Halliday, 1956). In man and monkey the transfer of antibodies to the foetus cannot be via the yolk-sac splanchnopleur and vitelline circulation since the yolk-sac is rudimentary. In horse, pig and sheep, where passive immunity is acquired via the colostrum, the yolk-sac is exposed to the uterine lumen for only a short time early in gestation. It gradually becomes separated from the uterine cavity by the growth of the allantois. Brambell (1958, 1961) drew attention to this diversity in the morphology of the foetal membranes in mammals and the accompanied diversity in function.

The transfer of antitoxins to the guinea-pig foetus about the time of the breaking of the decidua capsularis and the exposure of the yolk-sac splanchnopleur to the uterine cavity was determined by grinding the whole conceptus after the removal of the placenta. Some antitoxin was transferred to the foetus at about the 30th day of gestation. An approximate C.Q. of antitoxin in the foetal serum at 30 days of gestation was estimated by comparison with a ground foetus at an older age whose litter mates were bled normally and the

C.Q. values for antitoxin present in their sera were estimated. A C.Q. of about C.025 was calculated for antitoxin present in the foetal serum at 30 days of gestation. This finding means that some transfer of antitoxin from the mother to her foetus might have occurred before the breaking of the dicidua capsularis, which occurs at the 30th day. Anderson (1959) found that proteins passed into the yolk-sac cavity of foetal rats before the rupture of the dicidua capsularis. The arrangement of foetal membranes in guinea-pigs closely resembles that in the rat, so that a transfer of antibodies to foetal guinea-pig before the exposure of the visceral membrane of the yolk-sac to the uterine cavity may occur. The finding that antitoxin passes into the guinea-pig foetus before the 30th day of gestation, however, needs to be confirmed by a more delicate method than grinding; autoradiography or fluorescent microscopy for example are possible techniques for such a study.

Brambell and Mills (1947) and Brambell and Hemmings, with McCarthy and Kekwick (1949) found that maternal plasma proteins were present in the rabbit blastocyst on the eighth day of gestation. During this time the diameter of the blastocyst is nearly 1 cm., so about C.5 ml. of fluid could be collected. I had intended to study the transfer of antitoxins into the guinea-pig blastocyst just before implantation, but this study would

have been extremely difficult since the blastocyst of guinea-pigs at implantation measures only 0.09 x 0.1 x 0.056 mm. (Sansom and Hill, 1931). Also the most of implantation in the guinea-pig is different from that in the rabbit; it is interstitial in guinea-pigs and central in rabbits. This makes it even more difficult to wait until the blastocyst enlarges in size after implantation. Ovulation in the guinea-pig is spontaneous, this means that it is much more difficult to guarantee successful mating; so that a considerable number of animals would have had to be wasted in the hope of a few successes. It would have been very interesting to find whether maternal plasma proteins were present in the guinea-pig blastocyst before implantation.

No account of electrophoretic examination of guinea-pig foetal sera at various periods of gestation was found in the literature. Such a study along with immunological methods is planned for future work.

Our results show that passive transfer of immunity in guinea-pig is not augmented after birth. This is found by feeding new-born guinea-pigs with diphtheria or tetanus homologous and heterologous antitoxins of high values from 2 to 22 hrs. of age. Table 7 shows that very little absorption of antitoxin occurred at two i hours of age after the administration of 110 and 135 units of diphtheria antitoxin prepared in the guinea-pig and

rabbit respectively. A C.Q. of about C.CCC1 is found for diphtheria antitoxin in the newborn sera in both cases, this is considered insignificant compared to the amount of antitoxins transferred before birth in other experiments. No detectable absorption of antitoxins through the intestine is observed after this age. The slight absorption of antibodies by newborn guinea-pigs at two hours of age is interesting. Professor Oakley mentioned to me that newborn guinea-pigs are easily infected with Clostridium welchii type B by mouth in the first six hours of post natal life. Leissring and Anderson (1961b) found that "complete" agglutinins were absorbed by newborn guinea-pigs until the third day after birth. The absorption of "incomplete" antibodies was demonstrated by Leissring and Anderson as late as the 7th day of post natal life.

The concentration of diphtheria and tetanus antitoxins in the amniotic fluid of embryos of actively immunized guinea-pigs was much lower than that in the foetal sera. The C.Q. values of antitoxins present in the amniotic fluid did not exceed C.0043 (tables 1 and 2). There was a gradual increase in the concentration of antitoxins in the fluid as gestation advanced, however, from the 53rd day after mating to the 60th day, the oldest age tested, the concentration remained constant. This finding was in contrast to that of foetal serum where the

concentration of antitoxins increased at a faster rate in the few days before birth (figs. 13 and 14). Two C.Q. values of antitoxin in the amniotic fluid (encircled in fig. 14) were much higher than those of litter mates, this might be due to contamination with blood.

Barnes (1957) found that the percentage of diphtheria antitoxin in the amniotic fluid relative to that of the mother's serum increased from C.02 at 36 days to 0.33 at 68 days of gestation. If one calculates the C.Q. values of antitoxin present in the amniotic fluid of Barnes' experiments an increase from 0.0002 to C.003 is observed which is quite comparable to our results.

The low concentration of antitoxins in the amniotic fluid suggests that antibodies are not transmitted from the maternal circulation to that of the foetus via the amniotic fluid and foetal gut. Brambell, Hemmings, Henderson, Oakley and Rowlands (1951) eliminated this route for the transfer of antibodies to the foetal circulation in rabbits. Our suggestion is supported by the low concentration of antitoxin, whether homologous or heterologous, in the stomach contents of guinea-pig foetuses (table 6). The values of antitoxins present in the stomach contents were determined as far as material permitted; because these values were so low and the amounts of the samples were so small the tests were not carried to finality in the majority of cases. However,

it was found that a close correlation existed between the antitoxin values of the stomach contents to the values of the corresponding amniotic fluids. It was also found that the guinea-pig foetuses were unable to concentrate antitoxins in their stomachs as do rabbit foetuses (Brambell et al, 1951).

The appearance of antibodies in the amniotic fluid was thought to be via the uterine lumen and the non-vascular chorion and amnion (Brambell, Brierly, Halliday and Hemmings, 1954). The mechanism of entry of antibodies into the amniotic fluid was proposed by Brambell, Hemmings, Henderson and Oakley (1952) to be through "seepage" between the cells. The development of foetal membranes in the guinea-pig compared to that in the rabbit eliminates the possibility of transfer of antibodies from the uterine lumen via the chorion and the amnion since no chorion as such is present in the guinea-pig (fig. 12). Antibodies may enter the amniotic fluid of the guinea-pig foetus via the uterine lumen, yolk-sac and amnion traversing the following layers: the endoderm and mesoderm of the yolk-sac splanchnopleur into the exocoele and then traversing the mesoderm and ectoderm of the amnion into the amniotic cavity. The mechanism of entry of antitoxins into the amniotic cavity of guinea-pigs may also be by "seepage" between the cells as that of rabbits proposed by Brambell et al.

(1952). Unfortunately samples of exocoelomic fluid were so small that not even a single test could be performed in any case.

The entry of antitoxins into the foetal circulation is evidently selective in nature; selection was found to depend on the species of origin of the antitoxin. Intra-uterine injections of mixtures of homologous and heterologous antitoxins were made into time pregnant guinea-pigs at several stages of gestation. Table 4 gives evidence of selective transfer of antitoxins into the foetal circulation. Guinea-pig antitoxin whether diphtheria or tetanus, passed more readily than human, rabbit, horse or cow at all stages of gestation tested. The C.Q. values for homologous antitoxin in the foetal sera were always higher than those for heterologous antitoxins in the same experiment. Cow and horse antitoxins entered the foetal circulation much less readily than guinea-pig antitoxin. Human and rabbit antitoxins entered the foetal circulation only relatively, but significantly, less readily than guinea-pig antitoxin.

In experiment 296 however, the C.Q. for rabbit antitoxin in the foetal sera appeared to be twice as high as that for guinea-pig antitoxin. It was then thought that the bottle was wrongly labelled i.e., guinea-pig diphtheria antitoxin was wrongly labelled as

guinea-pig tetanus antitoxin and similarly rabbit tetanus antitoxin as diphtheria. To test whether diphtheria antitoxin present in the mixture injected into the uterine cavity of guinea-pig 296 was of guinea-pig or rabbit origin the following experiment was performed. A rabbit anti-guinea-pig serum was prepared by giving three intravenous injections of guinea-pig whole serum; these were repeated after a week and the rabbit was bled 6 days after the last injection. One ml. of the mixture that was injected into the uterine cavity of guinea-pig 296 was added to 5 mls. of rabbit anti-guinea-pig serum. The mixture was incubated at 37° for 1 hr and left at 4° overnight. The precipitate was centrifuged off and the supernatant fluid was then tested for the presence of diphtheria and tetanus antitoxins to test whether a reduction in the number of units of either diphtheria or tetanus had occurred during precipitation. It was found that the ratio of the number of units of tetanus antitoxin per ml. of mixture injected into the uterine cavity of guinea-pig 296 to that of diphtheria antitoxin in the same mixture was increased from 2:3 to 3:4 by precipitation with rabbit anti-guinea-pig serum i.e., the units per ml. of diphtheria antitoxin fell 20 per cent. The diphtheria antitoxin was therefore of guinea-pig origin. The mixture injected into the uterine cavity of guinea-pig 296

was therefore guinea-pig diphtheria antitoxin and rabbit tetanus antitoxin. In table 4, however, the corrected information was recorded.

The availability of three types of antitoxin made it possible to expose the foetal membranes to the homologous plus two heterologous antitoxins simultaneously. Diphtheria or tetanus antitoxin prepared in the guinea-pig was mixed with Cl. welchii α-antitoxin prepared in cow and with diphtheria or tetanus antitoxin prepared in human or rabbit. It was found that the homologous antitoxin i.e., guinea-pig antitoxin passed most readily and that rabbit, human, cow and horse antitoxins showed a C.Q. decreasing in that order. The experiments were ill planned to test the relative entry of the various heterologous antitoxins. This study can be made on guinea-pigs at a selected stage of pregnancy i.e., similar foetal age.

The foetal membranes were also selective in their admission of antitoxins present in the maternal circulation (table 3, guinea-pig 342). The homologous antitoxin passed more readily than the heterologous (human) antitoxin into the foetal circulation. The C.Q. for the homologous antitoxin in the foetal sera was higher in this experiment than in those where the antitoxin was injected into the uterine cavity. This is due to the longer exposure of the foetuses to the

antitoxin in the experiments where it was injected into the maternal circulation than those where it was injected into the uterine cavity. The antitoxin mixture injected into the uterus tends to drain from the cervix, so that the foetal membranes are exposed to antitoxin injected into the uterine cavity for a short time only.

Table 3 shows that rabbit and human antitoxin injected into the maternal circulation were transferred to that of the foetus in significant amounts after 24 hr. exposure. The C.Q. for rabbit antitoxin in the foetal sera was about 0.01 at 47 and 50 days of gestation; that for human antitoxin was about 0.04 at 50 days. These two antitoxins appeared in the foetal sera with a higher C.Q. when injected into the circulation than when injected into the uterine cavity of the mother. This can be explained by the longer exposure of the foetuses to the antitoxin when the material was injected into the maternal circulation. Horse refined or crude antitoxin entered from the maternal to the foetal circulation in trace amounts, after 24 hr. exposure at 60 days of gestation. No conclusion could be drawn from experiment 168 where horse unrefined antitoxin was injected into the maternal circulation because the amount injected was limited since the material contained preservative. The selective admission of antitoxin from the maternal into the foetal circulation must have occurred on the foetal and not on

the maternal side, for the maternal tissues were found to be non selective in admitting antitoxins from the uterine lumen into the maternal circulation. Table 5 shows that homologous and heterologous antitoxins injected into the uterine cavity of non-immune pregnant mothers entered the maternal circulation non-selectively; almost equal C.Qs. were obtained in the maternal serum for antitoxins prepared in guinea-pig, rabbit, man, horse and cow. This was observed at all stages of gestation tested (41 days to 56). Batty et al. (1954) found that antitoxins injected into the uterine cavities of pregnant rabbits reached the maternal circulation irrespective of the species of origin. Rabbit, man, guinea-pig, dog, horse and cow antitoxins passed equally readily from the uterine lumen to the maternal circulation of rabbits. The route of the transfer of antitoxin from the uterine cavity to the maternal circulation is not via the foetal circulation and placenta but directly through the uterine wall since the antitoxins were selectively admitted into the foetal circulation. Batty et al. (1954) have already reached this conclusion.

It is concluded that the yolk-sac splanchnopleur of the foetal guinea-pig selectively admits antitoxins to the foetal circulation. This selection depends on the species of origin of the antitoxin molecule; homologous antitoxin entered the foetal circulation

more readily than heterologous. Brambell, Hemmings, Henderson and Rowland (1950) found that the yolk-sac splanchnopleur of the rabbit embryo freely admitted to the foetal circulation homologous antibodies whereas the heterologous (equine and bovine) antibodies were almost, but not entirely, excluded. Brambell, Hemmings, Henderson and Oakley (1952) found that homologous antitoxins entered the foetal circulation at a rate at least fifty times greater than the heterologous (equine or bovine antitoxins). Batty et al. (1954) showed that the species in which the antitoxin was prepared affected the entry and concentration of the antitoxin into the foetal circulation of the rabbit. Antitoxins produced in rabbit, man, guinea-pig, dog, horse and cow entered the foetal circulation at decreasing concentrations in that order.

The selective transfer of antibodies from the maternal into the foetal circulation in man also depends on the species of origin of the antitoxin. This was discovered by Hartley (1948) to explain a case reported by Chesney (1945). A pregnant woman was treated with therapeutic antitoxin (refined antitoxin prepared in the horse) after an attack of diphtheria. Few days later she gave birth to twins who were nursed by the mother. One child contracted diphtheria from the mother and died from the disease, the other was treated with antitoxin and survived.

Hartley discovered that therapeutic antitoxin injected into the mother was not transferred to her foetuses partly because it was foreign and partly because it was refined.

Halliday (1955a) found that newborn rats selectively absorbed antibodies from the gut. This selection also depended on the species of origin of the antibody molecule. It was later found that some heterologous antisera interfered with the absorption of the homologous antisera by the gut of young rats (Halliday, 1958; Brambell, Halliday and Morris, 1958). Gamma-globulin was the serum fraction that interfered with the absorption of homologous and heterologous antibodies by the gut of young rats and that the albumin fraction had no effect (Brambell, Halliday and Morris, 1958). However, heterologous sera do not appear to interfere with the transmission of antibodies from the uterine cavity to the foetal circulation via the yolk-sac splanchnopleur, at any rate in rabbits (Batty et al., 1954). Our results did not show any interference in the transfer of antitoxins from the uterine cavity to the foetal circulation via the yolk-sac splanchnopleur by heterologous antisera.

The mechanism of selection in the rabbit was explained by Brambell and his colleagues. They suggested that both homologous and heterologous globulins were taken up

equally by the yolk-sac splanchnopleur cells and selection occurred when the proteins left the cells to enter the foetal circulation. (Brambell, Hemmings, Henderson and Oakley, 1952; Hemmings, 1956; Hemmings and Oakley, 1957; Hemmings, 1958).

The transfer of large molecules like proteins across biological membranes is a complicated phenomenon. It depends not only on the properties of the membrane itself but also on that of the molecule transferred across it. Selective permeability of a cell membrane is a qualitative as well as quantitative property in that it involves the absolute ability of the substance to cross the membrane and the relative rate at which it is transported.

The entry of antitoxins in the amniotic fluid is non-selective in nature. Our results presented in tables 3 and 4 show that homologous antitoxin did not pass into the amniotic cavity more readily than heterologous. In the majority of cases the antitoxin concentration present in the amniotic fluid was either below the limits of testing i.e., less than 0.001 for diphtheria and 0.002 for tetanus and for Cl. welchii α-antitoxin. Although the amount of amniotic fluid varied from foetus to foetus within and between litters this was not found to affect the concentration of antitoxin present in the fluid. No more antitoxin was found to be present in the smaller volume than in larger

ones.

Our results support Brambell, Hemmings, Henderson and Oakley's (1952) conclusion that entry of antitoxins in the amniotic fluid does not depend on the species of origin of the antibody molecule but depends on a process of "seepage" between the cells. This was later confirmed by Batty et al. (1954)

Hartley (1948, 1951) showed that pepsin-digested and refined diphtheria antitoxin prepared in guinea-pigs was transferred from the maternal to the foetal circulation in trace amounts. It was later shown (Barnes, 1957) that the route of transfer of antibodies from the maternal to the foetal circulation of guinea-pigs is via the uterine lumen, yolk-sac splanchnopleur and vitelline circulation. Our experiments were so designed that natural and pepsin-refined antitoxins prepared in the guinea-pig were injected simultaneously into the uterine lumen of pregnant guinea-pigs at the 45th day of gestation. Results show that pepsin-refined tetanus antitoxin is transmitted from the uterine cavity into the foetal circulation less readily than natural diphtheria antitoxin. The C.Q. for diphtheria antitoxin in the foetal sera was comparable to those experienced in experiments where both diphtheria or tetanus whole antitoxin prepared in guinea-pigs were mixed with various heterologous antitoxins and injected into the uterine

cavity of pregnant guinea-pigs (compare tables 4 and 8). Pepsin-refined tetanus antitoxin however, was transmitted to the foetal circulation at C.Q. of nearly two orders less than that for natural guinea-pig diphtheria antitoxin. This difference could be even more apparent if the real values for the transfer of pepsin-refined tetanus antitoxin was found. It was unfortunate that the amount of foetal serum was limited so the tests were not carried to finality and results were recorded as less than the lowest value tested. Our finding supports and adds to Hartley's that pepsin digested homologous diphtheria antitoxin was transferred to the foetal circulation less readily than natural antitoxin. Results also support the conclusion reached by Brambell, Hemmings, Henderson and Rowlands (1950) and Brambell, Hemmings and Oakley (1959) that the selective transmission of antibodies by the yolk-sac splanchnopleur depends on the species of origin and not on molecular size. Tetanus antitoxin prepared in guinea-pigs and refined with pepsin passed into the foetal circulation of guinea-pigs much less readily than the whole undigested diphtheria antitoxin also prepared in the guinea-pig. Brambell et al. (1959) found that in rabbits pepsin-digested diphtheria antitoxin prepared in rabbits was transmitted from the uterine cavity to the foetal circulation much less readily than natural

tetanus antitoxin also prepared in rabbits.

It was found that after pepsin-digestion of horse diphtheria antitoxin and rabbit anti-ovalbumin γ-globulin there was a decrease of their sedimentation constants from 7.2 to 5.7 and from 6.4 to 5.5 or 4.9 respectively (Petermann and Pappenheimer, 1941; Nisonoff, Wissler, Lipman and Woernley, 1960). If pepsin acts on the guinea-pig antibodies as it does on antibodies prepared in the horse and rabbit by reducing the molecular size then we can conclude that the part is transferred to the foetal circulation at a lower rate than the whole.

Brambell (1963) revived the observation first made by Hartley (1948) of the existence of a similarity between the transmission of passive immunity from mother to foetus and passive anaphylactic sensitization in guinea-pigs and of the dependability of both processes on the acceptibility of the antibody molecule to the cells. Antitoxins (indeed gamma globulins in general) prepared in guinea-pig, rabbit or man can readily sensitize guinea-pigs whilst those prepared in horse, ox, sheep, goat or pig do not. Also guinea-pig antitoxin loses its ability for passive anaphylactic sensitization and for passive transmission from mother to foetus after pepsin digestion (Hartley, 1951). Interference with the transmission of antibodies from

the gut to the circulation of suckling rats and mice by certain heterologous antisera is a phenomenon also observed in passive anaphylactic sensitization of guinea-pig (Brambell, 1963). Gamma globulin is the serum fraction that interferes in both processes; interference is due to competition for receptors on, or in, the cell.

The ability to detect the presence of antitoxins in the foetal circulation depends on the preservation of the antibody combining sites after pepsin-digestion. However, a part of the molecule that is essential for the transmission across the yolk-sac splanchnopleur into the foetal circulation is separated from the rest of the molecule during digestion. In rabbits γ-globulin and therefore, presumably for rabbit antitoxin this part is found to be fraction III of Porter (1959) (Brambell, Hemmings, Oakley and Porter, 1960).

Refined or whole antitoxin prepared in guinea-pigs passed equally readily from the uterine cavity to the maternal circulation of guinea-pigs at the 45th day of gestation (table 10). Nearly equal C.Qs. were obtained for the pepsin-refined and natural antitoxins in the maternal circulation. The presence of refined antitoxin in the maternal circulation is further support for the idea that antitoxins did not pass from the uterine lumen to the maternal circulation via the foetal circulation and placenta, since no refined antitoxin was detected

in the foetal circulation at the lowest value tested i.e., <0.01. Brambell, Hemmings and Oakley (1959) suggested that the pepsinized antitoxin may not reach the maternal circulation from the uterine cavity of pregnant rabbits at the 24th day of gestation as readily as natural antitoxin, however, they mentioned that the differences between the transfer of pepsinized and natural antitoxins are scarcely significant.

SUMMARY

1. The literature regarding the transfer of passive immunity from mother to young in some mammals is reviewed and summarized. A short note on the development and arrangement of foetal membranes and placentation is given in each case.

2. The transfer of diphtheria and tetanus antitoxins from actively immunized mothers to their young in the guinea-pig was studied. Results showed that the concentration quotients (C.Q.) - the ratio of the antitoxin concentration of the sample to that of the serum to which the foetus is exposed - for foetal serum increased from about C.C25 at 30 days of gestation to 2.95 at full term.

The transfer of antitoxins to the amniotic fluid is much lower than that to the foetal serum at all stages of gestation tested. The C.Q. for antitoxin in the amniotic fluid increased from about 0.00022 at 30 days of gestation to C.C043 (the highest) at 60 days.

3. The transfer of heterologous (rabbit, human and crude or refined horse antitoxins from the maternal to the foetal circulation was studied at various periods of gestation. Results showed that rabbit and human antitoxins are transferred to the foetal circulation

in significant amounts, but horse antitoxin, whether crude or refined, entered the foetal circulation in trace amounts. The concentration of antitoxins in the amniotic fluid is very low.

In one experiment the transfer of human tetanus antitoxin from the maternal to the foetal circulation was compared to the transfer of guinea-pig diphtheria antitoxin. The transfer of homologous antitoxin was slightly more than twice as great as for this heterologous antitoxin.

4. The selectivity of the foetal membranes to homologous and various heterologous antitoxins was studied by intra-uterine injection between 41 and 56 days of gestation. It was found that guinea-pig antitoxin entered the foetal circulation more readily than human and rabbit antitoxin and much more so than horse (crude or refined) and cow antitoxin at all periods of gestation tested. The concentration of antitoxins in the amniotic fluid was much lower than that in the foetal serum. Homologous and heterologous antitoxins entered the amniotic fluid at almost similar rates.

Antitoxins injected into the uterine cavity were found to enter the maternal circulation non-selectively.

5. The presence of antitoxins in the stomach content of some foetal guinea-pigs was studied. It was

found that the antitoxic values, whether homologous or heterologous, of the stomach contents did not exceed the antitoxic value of the correspondong amniotic fluids.

6. The transfer of antitoxins from the stomach to the circulation of newborn guinea-pigs was studied between 2 and 22 hours of age. It was found that homologous or heterologous (rabbit, man, horse and dog) antitoxins were not absorbed readily through the gut of newborn guinea-pigs. At 2 hrs of age guinea-pig and rabbit antitoxins were absorbed in trace amounts.

7. The transfer of natural and pepsin-refined homologous antitoxin from the uterine cavity to the foetus was studied on the 45th day of gestation. Natural antitoxin was transferred to the foetal circulation at levels comparable to those experienced earlier. Pepsin-refined antitoxin however, was not detected in the foetal circulation at the lowest level tested. Neither antitoxin was detected in the amniotic fluid or the stomach contents at the lowest values tested. The entry of each antitoxin into the maternal circulation was with almost equal C.Qs.

8. The results are discussed in relation to relevant work by other workers.

REFERENCES

AMOROSO, E.C. (1952). Chap. 15 in Marshall's Physiology of reproduction, 3rd ed. London: Longmans, Green.

AMOROSO, E.C. (1961). Histology of the placenta. Brit. Med. Bull. 17, 81-90.

ANDERSON, J.F. (1906). Simultaneous transmission of resistance to diphtheria toxin and hypersusceptibility to horse serum by the female guinea-pig to her young. J. Med. Res. 15, 259-260.

ANDERSON, J.W. (1959). The placental barrier to gamma-globulins in the rat. Amer. J. Anat. 104, 403-429.

ASDELL, S.A. (1946). Patterns of Mammalian reproduction. New York: Comstock Publishing Company Inc.

ASKONAS, B.A., CAMPBELL, P.M., HUMPHREY, J.H. and WORK, T.S. (1954). The source of antibody globulin in rabbit milk and goat colostrum. Bioch. J. 56, 597-601.

BANGHAM, D.R. and TERRY, R.J. (1957a). The absorption of ^{131}I-labelled homologous and heterologous serum proteins fed orally to young rats. Biochem. J. 66, 579-583.

BANGHAM, D.R. and TERRY, R.J. (1957b). The survival of globulins absorbed from the gut in suckling rats. Biochem. J. 66, 584-587.

BANGHAM, D.R., HOBBS, K.R. and TERRY, R.J. (1958) Selective placental transmission of serum proteins in the rhesus. Lancet (2), 351-354.

BANGHAM, D.R., INGRAM, P.L., ROY, J.H.B., SHILLAM, K.W.J. and TERRY, R.J. (1958). The absorption of ^{131}I-labelled serum and colostral proteins from the gut of the young calf. Proc. roy. Soc. B. 149, 184-191.

BANGHAM, D.R. (1960). The transmission of homologous serum proteins to the foetus and to the amniotic fluid in the rhesus monkey. J. Physiol. 153, 265-289.

BALFOUR, W.E. and COMLINE, R.S. (1962). Acceleration of the absorption of unchanged globulin in the newborn calf by factors in colostrum. J. Physiol. 160, 234-257.

BARNES, J.M. (1957). Observation on prenatal immunization. Thesis, University of London.

BARR, M., GLENNY, A.T. and RANDALL, K.J. (1949). Concentration of diphtheria antitoxin in cord blood and rate of loss in babies. Lancet (2), 324-326

BARR, M., GLENNY, A.T. and RANDALL, K.J. (1950). Diphtheria immunization in young babies. A study of some factors involved. Lancet (1), 6-10.

BARRICK, E.R., MATRONE, G. and OSBORNE, J.C. (1954). Effects of administrating blood serum constituents on gamma-globulin levels of baby pigs. Proc. Soc. exp. Biol. and Med. 87, 92-94.

BATTY, I., BRAMBELL, F.W.R., HEMMINGS, W.A. and OAKLEY, C.L. (1954). Selection of antitoxins by the foetal membranes of rabbits. Proc. roy. Soc. B, 142, 452-471.

BEARN, A.G. and PARKER, W.C. (1964). Some observation on transferrin. Iron metabolism; an international symposium by CIBA. Gross, F., Naegeli, S.R. and Philips, M.D. eds. Berlin, Gottingen and Heidelberg.

BENACERRAF, B., OVARY, Z., BLOCH, K.J. and FRANKLIN, E.C. (1963). Properties of guinea-pig 7S antibodies. I. Electrophoretic separation of the two types of guinea-pig 7S antibodies. J. exp. Med., 117, 937-949.

BESSIS, M. (1947). Etudes sur l'ictère Hémolytique expérimental par injection et ingestion d'antisérum. Rev. d'Hematol., 2, 114-146.

BLOCH, K.J., KOURILSKY, F.M., OVARY, Z. and BENACERRAF, B. (1963a). Properties of guinea-pig 7S antibodies. III. Identification of antibodies involved in complement fixation and hemolysis. J. exp. Med. 117, 965-981.

BLOCH, K.J., KOURILSKY, F.M., OVARY, Z. and BENACERRAF, B. (1963b). Properties of guinea-pig 7S antibodies. VI. Transmission of antibodies from maternal to foetal circulation. Proc. Soc. exp. Biol. Med. 114, 79-82.

BRAMBELL, F.W.R. and MILLS, I.H. (1947). Studies on sterility and prenatal mortality in wild rabbits. II. The occurrence of fibrin in the yolk-sac contents of embryos during and immediately after implantation. J. exp. Biol. 23, 332-345.

BRAMBELL, F.W.R., HEMMINGS, W.A. and ROWLANDS, W.T. (1947). Immunisation of the mammalian embryo. Lancet, (1), 759.

BRAMBELL, F.W.R. (1948). Prenatal mortality in mammals. Biol. Rev. 23, 370-407.

BRAMBELL, F.W.R., HEMMINGS, W.A., and ROWLANDS, W.T. (1948). The passage of antibodies from the maternal circulation into the embryo in rabbits. Proc. roy. Soc. B, 135, 390-403.

BRAMBELL, F.W.R., HEMMINGS, W.A., HENDERSON, M., PARRY, H.J. and ROWLANDS, W.T. (1949). The route of antibodies passing from the maternal to the foetal circulation in rabbits. Proc. roy. Soc. B, 136, 131-144.

BRAMBELL, F.W.R. and HEMMINGS, W.A. (1949). The passage into the embryonic yolk-sac cavity of maternal plasma proteins in rabbits. J. Physiol. 108, 177-185. With an addendum on Electrophoretic and Ultracentrifugal examination of rabbit blastocyst fluid by McCarthy, E.F. and Kekwick, R.A.

BRAMBELL, F.W.R., HEMMINGS, W.A., HENDERSON, M. and ROWLANDS, W.T. (1950). The selective admission of antibodies to the foetus by the yolk-sac splanchnopleur in rabbits. Proc. roy. Soc. B, 137, 239-252.

BRAMBELL, F.W.R., HEMMINGS, W.A. and HENDERSON, M. (1951). Antibodies and embryos. London:Athlone press.

BRAMBELL, F.W.R., HEMMINGS, G.P., HEMMINGS, W.A., HENDERSON, M. and ROWLANDS, W.T. (1951). The route by which antibodies enter the circulation after injection of immune serum into the exocoel of foetal rabbits. Proc. roy. Soc. B, 138, 188-195.

BRAMBELL, F.W.R., HEMMINGS, W.A., HENDERSON, M., OAKLEY, C.L. and ROWLANDS, W.T. (1951). The accumulation of antibodies in the stomach contents of foetal rabbits. Proc. roy. Soc. B, 138, 195-204.

BRAMBELL, F.W.R., HEMMINGS, W.A., HENDERSON, M. and OAKLEY, C.L. (1952). Selective and non-selective admission of various antitoxins into the foetal rabbit. Proc. roy. Soc. B, 139, 567-575.

BRAMBELL, F.W.R., HEMMINGS, W.A., HENDERSON, M. and KEKWICK, R.A. (1953). Electrophoretic studies of serum proteins of foetal rabbits. Proc. roy. Soc. B, 141, 300-314.

BRAMBELL, F.W.R., BRIERLY, J., HALLIDAY, R. and HEMMINGS, W.A. (1954). Transference of passive immunity from mother to young. Lancet, pp. 964-965.

BRAMBELL, F.W.R. and HALLIDAY, R. (1956). The route by which passive immunity is transmitted from mother to foetus in the rat. Proc. roy. Soc. B, 145, 170-178.

BRAMBELL, F.W.R. (1958). The passive immunity of the young mammal. Biol. Rev. 33, 488-531.

BRAMBELL, F.W.R., HALLIDAY, R. and MORRIS, I.G. (1958). Interference by human and serum fractions with the absorption of antibodies by suckling rats and mice. Proc. roy. Soc. B, 149, 1-11.

BRAMBELL, F.W.R., HEMMINGS, W.A. and OAKLEY, C.L. (1959). The relative transmission of natural and pepsin-refined homologous antitoxin from the uterine cavity to the foetal circulation in the rabbit. Proc. roy. Soc. B, 150, 312-317.

BRAMBELL, F.W.R., HEMMINGS, W.A., OAKLEY, C.L. and PORTER, R.P. (1960). The relative transmission of the fractions of papain hydrolyzed homologous γ-globulin from the uterine cavity to the foetal circulation in the rabbit. Proc. roy. Soc. B, 151, 478-482.

BRAMBELL, F.W.R. (1961). Problems concerning the transmission of immunity from mother to young. Proc. roy. Soc. Med. 54, 992-993.

BRAMBELL, F.W.R. (1963). Resemblances between passive anaphylactic sensitization and transmission of passive immunity. Nature, 199, 1164-1166.

BROOKS, M.E., STERNE, M. and WARRACK, G.H. (1957). A re-assessment of the criteria used for type differentiation of Clostridium perfringens. J. Path. Bact. 74, 185-195.

BRUCE-CHWATT, L.J. and GIBSON, F.D. (1956). Transplacental passage of Plasmodium berghei and passive transfer of immunity in rats and mice. Trans. roy. Soc. Trop. Med. Hyg. 50, 47-53

CAROLI, J. et BESSIS, M. (1947a). Recherches sur la cause de l'ictére grave familial des Muletons. Rev. d'hemat. 2, 207-228.

CAROLI, J. et. BESSIS, M. (1947b). Sur la cause et le traitment de l'ictére grave des Muletons nouveaunés. Comp. Rend. Acad. Sci. (Paris) 224, 969-971.

CHARLWOOD, P.A. and THOMSON, A. (1948). Electrophoretic patterns of lamb serum before and after transfer of colostrum. Nature, 116, p. 59.

CHESNEY, G. (1945). A note on the transmission of diphtheria antitoxin from mother to infant. Mon. Bull. Minist. Hlth. Lond., 4, 144-146.

CLARK, S.L. (1959). The ingestion of proteins and colloidal material by columnar absorptive cells of the small intestine in suckling rats and mice. J. Biophys. Biochem. Cyt. 5, 41-49.

COHEN, S.G. (1950). The placental transmission of antibodies and serum γ-globulin. J. Infec. Dis. 87, 291-298.

COMLINE, R.S., ROBERTS, H.E. and TITCHEN, D.A. (1951a) Route of absorption of colostrum globulin in the new-born animal. Nature, Lond., 167, 561-562.

COMLINE, R.S., ROBERTS, H.E. and TITCHEN, D.A. (1951b). Histological changes in the epithelium of the small intestine during protein absorption in the new-born animal. Nature, Lond., 168, 84-85.

CULBERTSON, J.T. (1938). Natural transmission of immunity against Trypanosoma lewisi from mother rats to their offspring. J. Parasit. 24, 65-82.

CULBERTSON, J.T. (1939a). The immunization of rats of different age groups against Trypanosoma lewisi by the administration of specific antiserum per os. J. Parasit. 25, 181-182.

CULBERTSON, J.T. (1939b). Transmission of resistance against Trypanosoma lewisi from a passively immunised mother rat to young nursing upon her. J. Parasit. 25, 182-183.

CULBERTSON, J.T. (1940). The natural transmission of immunity against Trypanosoma duttoni from mother mice to their young. J. Immunol. 38, 51-66.

CUMMINGS, J.N. and BELLVILLE, T.P. (1963). Studies on fetal physiology in the sheep. Transplacental passage of antibodies and techniques for repeated sampling of the fetal lamb in situ. Amer. J. Obstet. and Gynec. 86, 504 - 513.

DANCIS, J., SHAFRON, M. and MONEY, W.L. (1957). The transport of amnio acids and plasma proteins across the placenta in the guinea-pig. Amer. Med. Assoc. J. Dis. Child. 93, 8-9.

DANCIS, J., LIND, J., ORATZ, M., SMOLENS, J. and VARA, P. (1961). Placental transfer of proteins in human gestation. Amer. J. Obstet. and Gynec. 82, 167-171.

DEAN, D.J. (1952). Postnatal immunity in mouse encephalomyelitis. J. Immunol. 68, 549-557.

DEUTSCH, H.F. (1954). Fetuin: The mucoprotein of fetal calf serum. J. Biol. Chem. 208, 669-678.

DEUTSCH, H.F. and SMITH, V. (1957). Intestinal permeability to proteins in the newborn herbivore. Amer. J. Physiol. 191, 271-276

DUVAL, M. (1892). La placenta des rongeurs. Paris: Alcan.

EARLE, I.P. (1935). Influence of the ingestion of colostrum on the proteins of the blood sera of young foals, kids, lambs and pigs. J. Agric. Res. 51, 479-490.

EDELMAN, G.M. and BENACERRAF, B. (1962). On structural and functional relations between antibodies and proteins of the gamma-system. Proc. Nat. Acad. Sci. 48, 1035-1042

EHRLICH, P. (1892). Ueber Immunität durch Vererbung und Saugung. Z. Hyg. Infekt.Kr. 12, 183-203.

FAHLENER, L.W. (1912). On the transmission of immunity from mother to offspring. A study upon serum hemolysins in goats. J. Inf. Dis. 10, 332-368.

FOSTER, J.F., FRIEDELL, R.W., CARTON, W. and DIECKMANN, M.R. (1951). Electrophoretic studies on swine. III. Composition of baby pig plasma and sow's whey during lactation. Arch. Bioch. Biophys. 31, 104-112

FRANKLIN, E.C. and KUNKEL, H.G. (1958). Comparative levels of high molecular weight (19S) gamma-globulin in maternal and umbilical cord sera. J. Lab. Clin. Med. 52, 724-727

FREDA, V.J. (1962). Placental transfer of antibodies in man. Amer. J. Obstet. and Gynec. 84, 1756-1777

GELFAND, M.M., STREAN, G.J., PAVILANIS, V. and STERNBERG, J. (1960). Studies in placental permeability. Transmission of poliomyelitis antibodies, liproproteins and cholesterol in single and twin newborn infants. Amer. J. Obstet. and Gynec. 79, 117-133.

GITLIN, D., KUMATE, J., URRUSTI, J. and MORALES, C. (1964). The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. J. Clin. Invest. 43, 1938-1951.

GLENNY, A.T. and LLEWELLYN-JONES, M. (1931). The intracutaneous method of testing diphtheria toxin and antitoxin. J. Path. Bact. 34, 143-156.

GLENNY, A.T. and STEVENS, M. (1938). The laboratory control of tetanus prophylaxis. J. Roy. Army. Med. Cps. 70, 308-310

GORDON, J.E. and CURLEY, F.J. (1949). Induced latent infection and resultant active immunity to M II mouse encephalomyclitis virus in mice suckled by immune foster mothers. J. Infec. Dis. 85, 259-262.

GRAVES, J.H. (1963). Transfer of neutralizing antibody by colostrum to calves born of foot-and-mouth disease vaccinated dams. J. Immunol. 91, 251-256.

GROSSER, O. (1909). Die Wege der fötalen Ernährung. Samml. anat. physiol. Vortr. 3, 79-96.

GROSSER, O. (1927). Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere. München : Bergmann.

HALLIDAY, R. (1955a). The absorption of antibodies from immune sera by the gut of the young rat. Proc. roy. Soc. B, 143, 408-413.

HALLIDAY, R. (1955b). Prenatal and postnatal transmission of passive immunity to young rats. Proc. roy. Soc. B, 144, 427-430.

HALLIDAY, R. (1956). The termination of the capacity of young rats to absorb antibody from the milk. Proc. roy. Soc. B, 145, 179-185.

HALLIDAY, R. and KEKWICK, R.A. (1957). Electrophoretic analysis of the sera of young rats. Proc. roy. Soc. B, 146, 431-437.

HALLIDAY, R. (1958). The absorption of antibody from immune sera and from mixtures of sera by the gut of the young rat. Proc. roy. Soc. B, 148, 92-103.

HALLIDAY, R. (1959). The effect of steroid hormones on the absorption of antibody by the young rat. J. Endocrin. 18, 56-66.

HALLIDAY, R. and KEKWICK, R.A. (1960). The selection of antibodies by the gut of the young rat. Proc. roy. Soc. B, 153, 279-286.

HAMILTON, W.J., BOYD, J.D. and MOSSMAN, H.W. (1962). Human embryology. 3rd ed. Cambridge: Heffer, W. and sons.

HANSEN, R.G. and PHILLIPS, P.H. (1947). Studies on proteins from bovine colostrum. I. Electrophoretic studies on the blood serum proteins of colostrum-free calves and of calves fed colostrum at various ages. J. Biol. Chem. 171, 223-227.

HARMS, A.J. (1948). The purification of antitoxic plasmas by enzyme treatment and heat denaturation. Bioch. J. 42, 390-397.

HARTLEY, P. (1948). The behaviour of different types of homologous and heterologous diphtheria antitoxin when administered to pregnant guinea-pigs. Mon. Bull. Minist. Hlth. 7, 45-54.

HARTLEY, P. (1951). The effect of peptic digestion on the properties of diphtheria antitoxin. Proc. roy. Soc. B. 138, 499-513.

HEMMINGS, W.A. (1956). Protein selection in the yolk-sac splanchnopleur of the rabbit; the distribution of isotope following injection of ^{131}I-labelled serum globulin into the uterine cavity. Proc. roy. Soc. B, 145, 186-195.

HEMMINGS, W.A. and OAKLEY, C.L. (1957). Protein selection in the yolk-sac splanchnopleur of the rabbit: the fate of globulin injected into the foetal circulation. Proc. roy. Soc. B, 146, 573-579.

HEMMINGS, W.A. (1958). Protein selection in the yolk-sac splanchnopleur of the rabbit: the total uptake estimated as loss from the uterus. Proc. roy. Soc. B, 148, 76-83.

HEMMINGS, W.A. and JONES, R.E.C. (1963). The occurrence of macroglobulin antibodies in maternal and foetal sera of rabbits as determined by gradient centrifugation. Proc. roy. Soc. B, 157, 27-32.

HILL, K.J. (1956). Gastric development and antibody transference in the lamb, with some observation on the rat and guinea-pig. Quart. J. exp. Physiol. 41, 421-432.

HILL, K.J. and HARDY, W.S. (1956). Histological and histochemical observation of the intestinal cells of lamb and kids absorbing colostrum. Nature, 178, 1353-1354.

HOLFORD, F.E. (1930). The placental transmission of foreign proteins in rabbits. J. Immunol. 19, 177-216.

HOWE, P.E. (1921). An effect of the ingestion of colostrum upon the composition of the blood of new-born calves. J. Biol. Chem. 49, 115-118.

JAMESON, E., ALVAREZ-TOSADO, C. and SORTOR, H.H. (1942). Electrophoretic studies on newborn calf serum. Proc. Soc. exp. Biol. Med. 51, 163-165.

JOHNSON, P. and PIERCE, A.E. (1959). Ultracentrifugal and electrophoretic studies on neonatal calf sera and maternal colostrum. J. Hyg. 57, 309-320.

JO-KEIICHIRO. (1953). On the transmission of antibodies from mothers to their offsprings in experimental typhus fever, experiments in albino rats and guinea-pigs. Jap. J. Med. Sci. and Biol. 6, 299-310.

KABAT, E.A. and MEYER, M.M. (1961). Experimental immunochemistry, 2nd ed. Springfield. Ill: Charles C. Thomas.

KALLIS, N., DAGG, M.K. and STIMPFLING, J.H. (1963). Maternal transfer of isoantibody in mice. Transplantation 1, 535-545.

KERR, W.R. and ROBERTSON, M. (1943). A study of the antibody response of cattle to Trichomonas foetus. J. comp. Path. 53, 280-297.

KERR, W.R. and ROBERTSON, M. (1946). A study of the passively acquired antibody to Tr. foetus in the blood of young calves and its behaviour in agglutination tests and intradermal reactions. J. comp. Path. 56, 38-48.

KERR, W.R. and ROBERTSON, M. (1954). Passively and actively acquired antibodies for Tr. foetus in very young calves. J. Hyg. 52, 253-263.

KOSUNEN, T.U. and HALONEN, P. (1963). Transmission of maternal tetanus immunity to offspring in mice. Ann. Med. exp. Biol. Fenn. 41, 571-575.

KULANGARA, A.C. and SCHJEIDE, O.A. (1962). Foetal synthesis and transplacental passage of homologous serum proteins in the rabbit. Nature, 195, 811-812.

KULANGARA, A.C. and SCHECHTMAN, A.M. (1962). Passage of heterologous serum proteins from mother into foetal compartments in the rabbit. Amer. J. Physiol. 203, 1071-1080.

KULANGARA, A.C. and SCHECHTMAN, A.M. (1963). Do heterologous proteins pass from mother to fetus in cow, cat and guinea-pig? Proc. Soc. exp. Biol. Med. 112, 220-222.

KUTTNER, A. and RATNER, B. (1923). The importance of colostrum to the newborn infant. Amer. J. Dis. Child. 25, 413-434

LANDSTEINER, K. and WIENER, A.S. (1940). An agglutinable factor in human blood recognized by immune sera for Rhesus blood. Proc. Soc. exp. Biol. Med. 43, 223.

LARSON, B.L. and GILLESPIE, D.C. (1957). Origin of the major specific proteins in milk. J. Biol. Chem. 227, 565-573

LECCE, J.G., MORGAN, D.O. and MATRONE, G. (1964). Effect of feeding colostral and milk components on the cessation of intestinal absorption of large molecules (closure) in neonatal pigs. J. Nutr. 84, 43-48

LEISSRING, J.C. and ANDERSON, J.W. (1961a). The transfer of serum proteins from mother to young in the guinea-pig. I. Prenatal rates and routes. Amer. J. Anat. 109, 149-156.

LEISSRING, J.C. and ANDERSON, J.W. (1961b). The transfer of serum proteins from mother to young in the guinea-pig. III. Postnatal studies. Amer. J. Anat. 109, 175-182.

LEMÉTAYER, E., NICOL, L., JACOB, L., GIRARD, O. et CORVAZIER, R. (1946a). Immunité antitoxique colostrale du poulain issu de Juments Immunisées. Comp. Rend. Soc. Biol. 140, 854-856.

LEMÉTAYER, E., NICOL, L., JACOB, L., GIRARD, O. et CORVAZIER R. (1946b). Immunité antitoxique diaplacentaire du poulain issu de Juments immunisées. Comp, Rend. Soc. Biol. 140, 852-854.

LEVINE, P., NEWARK, N.J. and STETSON, R.E. (1939). An unusual case of intragroup agglutination. J. Amer. Med. Assoc. 113, 126-127.

LITTLE, R.B. and ORCUTT, M.L. (1922). The transmission of agglutinins of Bacillus abortus from cow to calf in the colostrum. J. exp. Med. 35, 161-171.

LOCKE, R.F., SEGRE, D. and MYERS, W.L. (1964). The immunologic behaviour of baby pigs IV. Intestinal absorption and persistence of 6.6S and 18S antibodies of ovine origin and their role in the immunologic competence of baby pigs. J. Immunol. 93, 576-583.

LONGSWORTH, L.G., CURTIS, R.M. and PEMBROK Jr. R.M. (1945). The electrophoretic analysis of maternal and fetal plasmas and sera. J. Clin. Invest. 24, 46-53

MANZALLO, A. and MARTINO, A.O.L. (1963). Estudio de transmision transplacentaria de la immunidad antidifterica y ensayos de vacunacion en el cobayo recien nacido. Rev. Asoc. Med. Arg. 77, 423-428.

MASON, J.H., DALLING, T. and GORDON, W.S. (1930). Transmission of maternal immunity. J. Path. Bact. 33, 783-797.

MILLOT, P. and GORIUS, J. (1950). Considérations sur le physio pathologie de l'ictére hémolytique du muleton- Rôle de la'llaitement. Importance du colostrum. Rev. Path. Comp. 50, 85-107.

MINETT, F.C. (1937). Staphylococcus antitoxin in the blood and milk of cows and other animals. J. Comp. Path. 50, 173-190.

MOORE, D.H., DU PAN, R.M. and BUXTON, C.L. (1949). An electrophoretic study of maternal, foetal and infant sera. Amer. J. Obstet. and Gynec. 57, 312-322.

MORGAN, E.H. (1964). Passage of transferrin, albumin and gamma globulin from maternal plasma to foetus in the rat and rabbit. J. Physiol. 171, 26-41.

MORRIS, B. (1950). The structure of the foetal yolk-sac splanchropleur of the rabbit. Quart. J. Microscopical. Sci. 91, 237-249.

MORRIS, I.G. (1958a). Experimentally induced haemolytic disease in young mice. J. Path. Bact. 75, 201-210.

MOSSMAN, H.W. (1926). The rabbit placenta and the problem of placental transmission. Amer. J. Anat. 37, 433-497.

MOSSMAN, H.W. (1937). Comparative morphogenesis of the foetal membranes and accessory uterine structures. Contr. to Embryol. 26, 133-247.

MYERS, W.L. and SEGRE, D. (1963). The immunological behaviour of baby pigs. III. Transplacental transfer of antibody globulin in swine. J. Immunol. 91, 697-700.

McALPINE, J.E. and RETTGER, L.F. (1925). Serological studies in bovine infectious abortion. J. Immunol. 10, 811-828.

McARTHUR, C.L. (1919). Transmissibility of immunity from mother to offspring in hog cholera. J. Infec. Dis. 24, 45-50.

McCARTHY, E.F. and McDOUGALL, E.I. (1949). Absorption of immune globulin by the young lamb after ingestion of colostrum. Nature, 164, 354.

McDIARMID, A. (1946). The transference of agglutinins for Brucella abortus from cow to calf and their persistence in the calf's blood. Vet. Rec. 58, 146-149.

MacFARLANE, R.G., OAKLEY, C.L. and ANDERSON, C.G. (1941). Haemolysis and the production of opalescence in serum and lecitho-vitellin by the α-toxin of Clostridium welchii. J. Path. Bact. 52, 99-103.

McGIRR, J.L. (1947). Colostral transmission of antibody substances from mother to offspring. Vet. J. 103, 345-56.

NELSON, J.B. (1932). The maternal transmission of vaccinal immunity in swine. J. exp. Med. 56, 835-840.

NELSON, J.B. (1934). The maternal transmission of vaccinal immunity II. The duration of active immunity in the sow and of passive immunity in the young. J. exp. Med. 60, 287-291.

NISONOFF, A., WISSLER, F.C., LIPMAN, L.N. and WOERNLEY, D.L. (1960). Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. Arch. Bioch. Bioph. 89, 230-244.

NORDBRING, F. (1957). The change in total nitrogen, electrophoretic pattern and antibody titre in porcine and bovine colostrum during the first days of lactation. Acta. Soc. Med. Ups. 62, 135-151.

NORDBRING, F. (1957). The failure of newborn premature infants to absorb antibodies from heterologous colostrum. Acta. Pediat. 46, 569-578.

NORDBRING, F. and OLSSON, B. (1957). Electrophoretic and immunological studies on sera of young pigs. I. Influence of ingestion of colostrum on protein pattern and antibody titre in sera from suckling pigs and the changes throughout lactation. Acta. Soc. Med. Ups. 62, 193-212.

NORDBRING, F. and OLSSON, B. (1958a). Electrophoretic and immunological studies on sera of young pigs. II. The effect of feeding bovine trypsin inhibitor with porcine colostrum on the absorption of antibodies and immune globulins. Acta. Soc. Med. Ups. 63, 25-40.

NORDBRING, F. and OLSSON, B. (1958b). Electrophoretic and immunological studies on sera of young pigs. III. Transfer of protein fractions and antibodies to the newborn pigs by ingestion of porcine serum with a study of the effect of bovine trypsin inhibitor. Acta. Soc. Med. Ups. 63, 41-52.

OAKLEY, C.L. and WARRACK, G.H. (1941). Factors affecting the activity of the α-toxin of Clostridium welchii. J. Path. Bact. 53, 335-370.

OAKLEY, C.L. (1954). Bacterial toxins. Ann. Rev. Microbiol. 8, 441-428.

OLSSON, B. (1959a). Studies on the formation and absorption of antibodies and immune globulins in piglets. II. The intestinal absorption of antibodies and immune globulins by new-born piglets after the administration of bovine colostrum. Nord. Vet. Med. 11, 375-390.

OLSSON, B. (1959b). Studies on the formation and absorption of antibodies and immune globulins in piglets. III. The intestinal absorption of heterologous antibodies and serum proteins in newborn piglets. Nord. Vet. Med. 11, 441-460.

CRCUTT, M.L. and HOWE, P.E. (1922). The relation between the accumulation of globulins and the appearance of agglutinins in the blood of new-born calves. J. exp. Med. 36, 291-308.

OSBORN, J.J., DANCIS, J. and JULIA, J.F. (1952a) Studies of the immunology of the new-born infant. I. Age and antibody production. Pediatrics, 9, 736-744.

OSBORN, J.J., DANCIS, J. and JULIA, J.F. (1952b). Studies of the immunology of the new-born infant. II. Interference with active immunization by passive transplacental circulating antibody. Pediatrics 10, 328-334.

OSBORN, J.J., DANCIS, J. and ROSENBERG, B.V. (1952). Studies of the immunology of the new-born infant. III. Permeability of the placenta to maternal antibody during fetal life. Pediatrics 10, 450-456.

OVARY, Z., BENACERRAF, B. and BLOCK, K.J. (1963). Properties of guinea-pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis. J. exp. Med. 117, 951-964.

PANSE, M.V. and DUTTA, N.K. (1964). Cholera vaccines and placental transmission of antibodies. J. Immunol. 93, 243-245.

PAYNE, L.C. and MARSH, C.L. (1962). Gamma globulin absorption in the baby pig: The nonselective absorption of heterologous globulins and factors influencing absorption time. J. Nutr. 76, 151-158.

PEDERSEN, K.O. (1944). Fetuin, a new globulin isolated from serum. Nature, 154, 575.

PEDERSEN, K.O. (1945). Ultracentrifugal studies on serum and serum fractions. Uppsala: Almquist and Wiksells.

PEDERSEN, K.O. (1947). Ultracentrifugal and electrophoretic studies of fetuin. J. phys. Chem. 51, 164-171.

PETERMANN, M.L. and PAPPENHEIMER, A.M. (1941). The ultracentrifugal analysis of diphtheria proteins. J. Phys. Chem. 45, 1-9.

PIERCE, A.E. (1955). Electrophoretic and immunological studies on sera from calves from birth to weaning. I. Electrophoretic studies. J. Hyg. Camb. 53. 247-60.

PIERCE, A.E. (1955b). Electrophoretic and immunological studies on sera from calves from birth to weaning. II. Electrophoretic and serological studies with special reference to the normal and induced agglutinins to Trichomonas foetus. J. Hyg. 53. 261-275.

PIERCE, A.E. and FEINSTEIN, A. (1965). Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland for maternal plasma to colostrum. Immunology 8, 106-123.

POLSON, A. (1943). Variation of serum composition with the age of horses as shown by electrophoresis. Nature, 152, 413-414.

PORTER, R.R. (1959). The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. Bioch. J. 73, 119-126

QUINLIVAN, L.G. (1964a). Transplacental passage of gamma-globulin ^{131}I in the rat. Amer. J. Physiol. 207, 782-786.

QUINLIVAN, L.G. (1964b). Antepartum and postpartum transfer of gamma globulin-^{131}I in the rat. Amer. J. Physiol. 207, 787-788.

QUINLIVAN, L.G. (1964c). Fetomaternal transfer of ^{131}I-gamma globulin in the rat. Amer. J. Obstet. and Gynec. 88, 415-420.

QUINLIVAN, L.G., CONTOPOULOS, A.N. and MASCUREDIS, S.P. (1964). Fetomaternal and feto-fetal transfer of ^{131}I-gamma globulin in the rat. Proc. Soc. exp. Biol. Med.115, 49-51.

RATNER, B., JACKSON, H.C. and GRUEHL, H.L. (1927). Transmission of protein hypersensitiveness from mother to offspring II. The role of colostrum. J. Immunol. 14, 267-274

RATNER, B., JACKSON, H.C. and GRUEHL, H.L. (1927c). Transmission of protein hypersensitiveness from mother to offspring. III. The role of milk. J. Immunol. 14, 275-290.

RATNER, B., JACKSON, H.C. and GRUEHL, H.L. (1927d). Transmission of protein hypersensitiveness from mother to offspring IV. Passive sensitization in utero. J. Immunol. 14, 291-302.

REMINGTON, C. and PICKFORD, J.A. (1947). Pre- and post-natal development of immunity; serum-albumin and serum globulin levels in maternal and cord bloods of premature infants. Lancet, p. 781.

REYMANN, G.C. (1920). On the transfer of so-called normal antibodies from mother to offspring. I. *Agglutinins.* J. Immunol. 5, 227-238.

RODOLFO, A. (1934). A study of the permeability of the placenta of the rabbit to antibodies. J. exp. Zool. 68, 215-233.

RÖMER, P.H. AND SAMES, T. (1909). Zur Bestimmung sehr Kleiner Mengen Diphtherie-antitoxin. Z. ImmunForsch. 3, 344-351.

ROWLANDS, I.W. (1949). Post-partum breeding in the guinea-pig. J. Hyg. 47, 281-287.

RUTQVIST, L. (1958). Electrophoretic patterns of blood serum from pig fetuses and young pigs. Amer. J. Vet. Res. 19, 25-31.

SAN CLEMENTE, C.L. and HUDDLESON, I.F. (1943). Electrophoretic studies of the proteins of bovine serums with respect to Brucella. Mich. State. Coll. Agric. exp. Stn. Tech. Bull. 182, 3-44.

SAMSOM, G.C. and HILL, J.P. (1931). Observations on the structure and mode of implantation of the blastocyst of Cavia. Trans. Zool. Soc. Lond. 21, 295-354.

SCHECHTMAN, A.M. and ABRAHAM, K.C. (1958). Passage of serum albumins from the mother to the foetus. Nature, 181, 120-121.

SCHNEIDER, L. und SZATHMÁRY (1939a). Ueber die Immunität des neugeborenen Lammes. Z. ImmunForsch 95, 169-177.

SCHNEIDER, L. und SZATHMÁRY (1939b). Ueber die Immunität des neugeborenen Hundes. Z. ImmunForsch 95, 177-188.

SCHNEIDER, L. und SZATHMÁRY (1939c). Ueber die Immunität des neugeborenen Kaninchens. Z. ImmunForsch 95, 189-200.

SCHNEIDER, L. und SZATHMÁRY (1940). Ueber die Immunität der neugeborenen Meerschweinchen. Z. ImmunForsch. 98, 24-30.

SCLARE, G. and TAYLOR, G. (1961). Experimental thyroditis and the placental transfer of auto-antibodies. J. Path. Bact. 82, 29-44.

SEGRE, D. and KAEBERLE, M.L. (1962a). The immunologic behaviour of baby pigs. I. Production of antibodies in three-week-old pigs. J. Immunol. 89, 782-789.

SEGRE, D. and KAEBERLE, M.L. (1962b). The immunologic behaviour of baby pigs. II. Production of antibodies in newborn pigs. J. Immunol. 89. 790-793.

SELLE, R.M. (1922). Changes in the vaginal epithelium of the guinea-pig during the oestrous cycle. Amer. J. Anat. 30, 429-443.

SMITH, Th. and LITTLE, R.B. (1922). The significance of colostrum to the new-born calf. J. exp. Med. 36, 181-198.

SMITH, Th. (1930). The immunological significance of colostrum. I. The relation between colostrum, serum and the milk of cows normal and immunized towards B. coli. J. exp. Med. 51, 473-481.

SMITH, Th. and LITTLE, R.B. (1930). The immunological significance of colostrum. II. The initial feeding of serum from normal cows and cows immunized towards B. coli in place of colostrum. J. exp. Med. 51, 483-492.

SMITH E.L. (1946). The immune proteins of bovine colostrum and plasma. J. Biol. Chem. 164, 345-350.

SMITH E.L. and HOLM, A. (1948). The transfer of immunity to the newborn calf from colostrum. J. Biol. Chem. 175, 349-357.

SPEER, V.C., BROWN, K., QUINN, L. and CARTON, D. (1959). The cessation of antibody absorption in the young pig. J. Immunol. 83, 632-639

STÄUBLI, C. (1903). Zur Frage des ueberganges der Typhusagglutinine von der Mutter auf den Fötus. Centbl. Bakt. (originale) 33, 458-461.

STERZL, J., KOSTKA, J., MANDEL, L., RIHA, I. and HOLUB, M. (1960). Development of the formation of γ-globulin and normal and immune antibodies in piglets reared without colostrum. Proc. Symp. Prag. (1959). Mechanism of antibody formation: Prag.

STOCKARD, C.R. and PAPANICOLAOU, G.N. (1917). The existence of a typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes. Amer. J. Anat. 22, 225-283.

STOCKARD, C.R. and PAPANICOLAOU, G.N. (1919). The vaginal closure membrane, copulation and the vaginal plug in the guinea-pig, with further consideration of the oestrous rythm. Biol. Bull. 37, 222-245.

TERRY, R.J. (1956). Transmission of antimalarial immunity (Plasmodium berghei) from mother rats to their young during lactation. Trans. roy. Soc. Med. Hyg. 50, 41-46.

THOMPSON, R. and MEYERS, F.P. (1950). Passive transfer of immunity to Lansing poliomyelitis virus from actively immunized mothers to young mice. Amer. J. Hyg. 52, 213-221.

THORPE, F. and GRAHAM, R. (1933). The persistence of Brucella agglutinins in calves of reactor cows. J. Amer. Vet. M.A. 82, 871-874.

TIMMERMAN, W.A. (1931). Zur Frage der Uebertragung des Typhus "H" - und "O" - agglutinin von Mutter auf kind. Z. ImmunForsch. 70, 388-399.

VAHLQUIST, B. and HÖGSTEDT, C. (1949). Minute absorption of diphtheric antibodies from the gastrointestinal tract in infants. Pediatrics 4, 401-405.

VAHLQUIST, B. (1960). Neonatal immunity. Amer. Med. Assoc. J. Dis. Child. 99, 729-734.

WASZ-HOCKERT, O., WAGER, O., HAUTALA, T. and WIDHOLM, C. (1956). Transmission of antibodies from mother to foetus. A study of the diphtheria antitoxin level in the newborn with oesophageal atresia. Ann. Med. exp. Biol. Fenn. 34, 444-446.

WELLMANN, G., SCHWIETZER, C.H. und LIEBKE, H. (1961). Beobachtungen über die unterschiedliche Fähigkeit von Sauen, Antikörper mit der Kolostralmilch zu übertragen. Zbl. Bakt. (originale) 183, 217-224.

WELLMANN, G., LEIBKE, H. und ENGEL, H. (1962). Antikörpergehalt der Saugmilch und Antikörperresorption durch die Ferkel zu verschiedenen zeiten nach der Geburt. Zbt. Bakt. (originale) 185, 215-229.

WELLMANN, G. und ENGEL, H. (1963). Immunbiologische untersuchungen an künstlich aufgezogenen Ferkeln. 3. Über die Fähigkeit neugeborener Ferkel, Antikörper und γ-globulin aus der Kolostralmilch zu resorbieren. Zbl. Bakt. (originale) 190, 408-414.

WIENER, A.S. (1948). Rh factor in immunological reactions. Ann. Allergy 6, 293-304.

WILLIAMS, H.E. (1961). Colostral antibody to rabies in cattle vaccinated with HEP Flury strain of the virus. Amer. J. Vet. Res. 22, 902-905.

WINKLER, E.G., FITZPATRICK, J.G. and FINNERTY, J.J. (1958). The permeability of the rabbit placenta to homologous albumin. Amer. J. Obstet. and Gynec. 76, 1209-1213.

WISLOCKI, G.B., DEANE, H.W. and DEMPSEY, E.W. (1946). The histochemistry of the rodent's placenta. Amer. J. Anat. 78, 281-345.

WISLOCKI, G.B. and DEMPSEY, E.W. (1955). Electron microscopy of the placenta of the rat. Anat. Rec. 123, 33-63.

YOUNG, W.C. (1937). The vaginal smear picture, sexual receptivity and the time of ovulation in the guinea-pig. Anat. Rec. 67, 305-325.

YOUNG, G.A. and UNDERDAHL, N.B. (1950). Neutralization and hemagglutination inhibition of swine influenza virus by serum from suckling swine and by milk from their dams. J. Immunol. 65, 369-373.

ZUELZER, W.W. and KAPLAN, E. (1954). ABO heterospecific pregnancy and hemolytic disease. II. Patterns of A and B isoantibodies in the cord blood of normal infants. Amer. J. Dis. Child. 88, 179-192.