A STUDY OF SOME BLOOD CELLULAR ANTIGENIC FACTORS AND ISO-IMMUNISATION IN THE PIG

By

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TO MY WIFE

CONTENTS

PREFACE

The work described in this dissertation was carried out in the Meat and Livestock Commission's Pig Blood Group Research Unit attached to the Department of Animal Health, Royal(Dick) School of Veterinary Studies, University of Edinburgh, and supervised by Dr. P. Imlah, M.Sc., Ph.D., M.R.C.V.S.

Parts of the results have already been published in brief form and reprints of the papers are included in Appendix VII. Part of the work was also presented as a paper at the XIIth European Conference on Animal Blood Groups and Biochemical Polymorphism, Budapest, 1970, and will be published in the Proceedings of that Conference.

Fig.1.2, published in one of the papers, was subsequently published in "The transmission of passive immunity from mother to young" by F.W. Rogers Brambell, North-Holland Publishing Company, Amsterdam/London, 1970, p.291.

This is a record of original work carried out by the author and has not been submitted in any form to any other University.

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Summary

It has been shown for the first time that iso-immunisation of sows by foetal red cell antigens can occur. By studies on two sows in a minimal disease pig unit, it was demonstrated that iso-antibodies were produced to the blood group factor, Ea, after giving birth to Ea positive piglets but not after giving birth to Ea negative piglets alone. Complete and reliable life histories of both sows were available and the possibility of previous injections of pig red cells in any form was precluded.

Further studies were carried out on two Ea negative, Kb negative sows which were mated to an Ea positive, Kb positive boar. In one of these, anti Ea was boosted after farrowing as in the previous two cases, while in the other the anti-Ea titre rose about six weeks before parturition. In addition anti-Kb was produced in both at the same time as anti-Ea. A subsequent mating of the first of these to an Ea negative, Kb positive boar resulted in the boosting of the anti-Kb titre but not the anti-Ea titre, again after parturition. Likewise on mating the second sow to an Ea negative, Kb negative boar, there was little effect on the anti-Ea and anti-Kb titres either throughout pregnancy or after parturition.

Two Ea negative gilts and a further Ea negative sow, all of which had no evidence of red cell iso-antibodies in their sera were mated to an Ea positive boar. Anti-Ea was detected in the sera of two of them for the first time after the subsequent parturition; the third remained negative.

On examining the sera from eighty sows and sixty-seven

boars in the routine typing service carried out by the Blood Group Research Unit, no antibodies other than anti-A were found in the boars' sera while, in twenty-six per cent of the sows' sera, red cell iso-antibodies were found as well as anti-A. All these sows were bred to boars of the same breed as themselves. Similarly, the sera of fifty per cent of twenty-four sows which had produced litters affected with thrombocytopenic purpura and which were mainly bred to boars of different breeds than themselves, had iso-antibodies present.

Of these iso-antibodies, anti-Ea and anti-Eb were the most prevalent and also present at the highest titres. Anti-Ee, anti-Fa, anti-Ka, anti-Kb, anti-Kd, anti-La and anti-Lg were also found but usually at only low titres.

The effect of anti-Ea on the red cells of piglets in four litters was studied. Although the antibody was absorbed from the colostrum by all the piglets, it had little effect on their haematological pictures and no differences were noted between Ea positive and Ea negative piglets in the same litter.

All these results support further the hypothesis that iso-immunisation of sows by incompatible foetal antigens does occur. In support of this hypothesis a condition of piglets known as thrombocytopenic purpura has been reported in several Scandinavian countries as well as in the United Kingdom. It is considered that iso-immunisation of the sow by thrombocytes of the foetuses takes place <u>in vitro</u> or at parturition resulting in the destruction of thrombocytes in the neonatal piglets after absorption of antibodies from

their dam's colostrum. Four litters affected by this condition were studied in detail and clinical symptoms similar to that described by the other workers were noted.

Two gilts, non-affected members of a litter which suffered from the condition, were mated to a boar, an affected survivor of a similar litter. During pregnancy the gilts were injected with pure thrombocyte suspensions from the boar. The piglets produced from these matings had normal thrombocyte counts at birth but a proportion of them developed thrombocytopenia and purpura similar to that seen in natural cases within a few hours of receiving colostrum. However, despite the fact that this condition was observed in three litters from each of these gilts, a secondary thrombocytopenia at ten to fourteen days of age, characteristic of the naturally occurring condition, was only observed in two piglets in one litter.

Of all techniques investigated the antiglobulin consumption test was found to be the most satisfactory for the detection of thrombocyte antigen/iso-antibody reactions. Using this, the thrombocytes of affected and non-affected piglets in the litters experimentally affected with thrombocytopenic purpura, were tested against their dam's serum. A correlation was demonstrated between the serological reactions of the piglets' thrombocytes and the clinical signs of purpura and thrombocytopenia.

INTRODUCTION

Introduction

The immunological aspects of mammalian pregnancy have attracted a great deal of attention. The foetus, having inherited antigens from its father is, to a certain degree, immunologically incompatible with the mother and is, in effect, a homograft within her. The reasons for it not being rejected by the mother still remain obscure. Undoubtedly the barrier presented by the placenta plays a big part in this and also protects the foetus from maternal antigens it may not have inherited, as it is now known that many mammalian foetuses are immunologically competent whilst in utero (Binns, 1968).

Serological reactions in the mother to foetal antigens have been found to occur in both man and other animals and antibodies to the antigens of the blood cellular elements, especially red cells and thrombocytes, have been frequently described. These give rise to foetal and neonatal diseases of varying severity when the maternal antibodies are transmitted to offspring possessing the corresponding antigens.

Haemolytic disease of newborn children has been described for a long time and Roberts (1957) has comprehensively reviewed the historical aspects dating back to the seventeenth century. After the first observation of Landsteiner in 1900 when he reported

that the sera of some of his colleagues agglutinated the red cells of others, great interest was stimulated in blood groups of man especially once it was established in 1910 by von Dungern and Hirszfeld that they were inherited in a Mendelian fashion. Therefore, it is surprising that it was not until 1939 that the work of Levine and Stetson correlated haemolytic disease of newborn babies with blood group incompatibility. Almost simultaneously Landsteiner and Wiener (1940) discovered the Rh blood groups of man and blood grouping entered the field of scientific medicine.

Since then literally thousands of papers have been published on the subject and haemolytic disease of newborn babies is now well documented. Immunization of the mother has been shown to take place by blood group antigens of the foetus but, although blood group incompatibility is quite common, immunization of the mother does not always take place and hence haemolytic disease does not always ensue. Further, the antigens from the foetus must be capable of initiating a strong antibody response in the mother. Usually one or more of the first born children are not affected and the disease occurs in subsequent offspring, the antibody response in the mother being apparently boosted at successive pregnancies.

As antibodies can cross the human placenta, foetuses may be affected <u>in utero</u>, leading to foetal death in

extreme cases. Mostly the symptoms are mild at this time and become more apparent in the neonatal period when the haemolytic anaemia may be so mild as to pass almost unnoticed, or, as in the majority of cases, severe and acute and accompanied by jaundice.

Following on the description of haemolytic disease in newborn babies, a similar condition was reported in horses and mules and eventually, in the early nineteen fifties, in piglets. In all of these the foetuses were protected from the antibodies in utero and became affected after first sucking colostrum. The pathogenesis of the condition in horses and mules was found to be similar to that in humans in that immunization of the mare followed on previous pregnancies in which foals with incompatible red cell antigens had been carried. In pigs, on the other hand, two possibilities existed. English workers clearly showed the correlation between haemolytic disease of newborn piglets and vaccination with crystal violet swine fever vaccine while Hungarian ones described the syndrome as occurring without artificial immunization of the sow. The former idea completely overshadowed the latter which therefore was quietly forgotten.

A great deal of interest in blood grouping in pigs was generated after this especially as it was taken up as a tool by the geneticists to aid in their quest for pigs with better production traits. The more optimistic

hoped to find a correlation between blood group antigens and these characters to help in the selection of the best animals but it was mainly as a means of checking the parentage of animals being sent for performance and progeny testing that blood grouping found a place in animal breeding. Laboratories were formed in most European countries and blood grouping became associated with animal breeding with the result that the disease aspects were neglected.

Meanwhile a neonatal condition of babies manifest by thrombocytopenia and purpura had been noted and a similar pathogenesis to haemolytic disease of the newborn had been put forward. Antibodies produced to the foetal thrombocyte antigens in the mother, on gaining access to the foetus, destroyed a varying amount of its thrombocytes and upset its haemostatic mechanism resulting in a haemorrhagic anaemia. When a similar condition was reported in piglets in the early nineteen sixties, the same theory for its pathogenesis was put forward. Unlike the cases of haemolytic anaemia earlier reported, no means of artificial immunization could be found in this case and the whole question of iso-immunization of the sow by foetal blood group antigens was reopened.

Review of Literature

a) Iso-antibodies to porcine red cell antigens

It has been known for a long time that the serum of one individual may agglutinate the red blood cells of another of the same species, a phenomenon which Ehrlich termed iso-agglutination. In 1913 Fishbein described experiments on iso-agglutination in pigs along with cattle, sheep, rabbits, frogs and dogs. Although the blood and serum of 60 pigs were studied he illustrated his findings by a table of only 20 specimen results (Table I).

It is interesting to note that only a few of these 20 sera gave identical results and, in fact, most gave a mixture of reactions with different cells. Only 2 of the 20 sera were completely negative and, put into present day nomenclature for pig blood groups, these would be expected to be of group Aa. However, it is surprising that the cells from one of these animals were not agglutinated by any of the other 19 sera while the other was agglutinated by only one sample. No definite pattern emerged but it would seem from the wide variation in the reactions presented and by the fact that the blood from a number of animals was agglutinable as well as agglutinating that antibodies other than anti-A were present in the 20 pig sera examined. The origin and history of the animals was not given.

The findings of Weszeczky (1920) confirmed Fishbein's

TABLE I

Isoagglutination in Swine

Corpuscles	Serum																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I	0	0	0	0	0	0	0	0	0	+	0	+	+	+	+	0	0	0	+	0
II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IV	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
V	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
VI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VII	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0
VIII	+	0	0	0	0	0	0	0	0	0	0	0	+	0	+	+	+	0	+	0
IX	0	0	0	0	+	0	0	0	0	0	+	0	0	0	+	0	0	0	+	0
X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XI	0	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0
XII	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
XIII	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
XIV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XV	+	0	0	0	+	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0
XVI	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0
XVII	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
XVIII	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XIX	+	0	0	0	0	+	0	+	+	+	+	+	0	+	+	+	0	0	0	+
XX	+	+	+	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	+	0

after Fishbein (1913)

TABLE II

Rbc.	Pigs	Serum										
		1	2	3	4	5	6	7	8	9	10	11
1	Gilt	-	-	-	-	-	-	-	-	-	-	-
2	Pregnant gilt	+	-	+	+	+	-	-	+	+	+	-
3	Gilt	-	-	-	-	-	-	-	-	-	-	-
4	Suckling gilt	-	-	+	-	-	-	-	-	-	-	-
5	Castrate	-	-	-	-	-	-	-	-	-	-	-
6	"	-	-	+	-	-	-	-	+	+	+	-
7	Gilt	-	-	-	-	+	-	-	+	+	+	-
8	Castrate	-	-	-	-	-	-	-	-	-	-	-
9	"	-	-	-	-	-	-	-	-	-	-	-
10	"	-	-	-	-	-	-	-	-	-	-	-
11	Suckling pig	-	-	-	-	-	-	-	-	-	-	-

after Weszeczky (1920)

work but they demonstrated a more regular pattern of reactions (Table II). Only 11 animals were examined in this case and, of these, only four (2, 6, 7 & 11) had no antibodies in their sera and might be considered as being potentially of group Aa and, in fact, three of these (2, 6 & 7) are the ones whose red cells are all agglutinated by sera 8, 9 & 10 and less consistently by sera 1, 3, 4 & 5. Allowing for the lack of sensitivity of the agglutination test used the eleven animals could presumably be grouped for the A system as follows:

Pig No.	1	2	3	4	5	6	7	8	9	10	11
A system	Ao	Aa	Ao	Ao	Ao	Aa	Aa	Ao	Ao	Ao	Ao or i

On this theory the only aberrant reaction was that between the red cells of 4 and the serum of 3. Here again may be evidence for the presence of antibodies other than anti-A. Again the history of the animals used was not available and all that was stated is that the blood was obtained from slaughter house animals.

However, the first direct evidence that Ao cells might be antigenic was given by Szymanowski and Wachlerowna in 1926 when they showed that pigs vaccinated against swine fever occasionally had antibodies against A negative cells. They had at the same time (Szymanowski, Stetkiewicz and Wachlerowna, 1926) discovered a regular pattern of reactions

and classified pigs according to the presence or absence of the blood group factor A and also on the basis of the corresponding antibody in the serum and published a comprehensive report of their findings in 1927 (Szymanowski and Wachlerowna, 1927).

In the ensuing years most interest centred on the A system in pigs and its inheritance and on natural hetero-antibodies to red cells (Kayser, 1929; Schermer, Kayser and Kaempffer, 1930; and Kaempffer, 1932a). In his paper on the inheritance of blood groups in pigs, Kaempffer recognised the fact that other antibodies might be present and has adequate controls to back this up. He cites Winter (1930) who found, apart from A, another blood type which he called B and its antibody anti-B in American pigs. In a second report the same year (Kaempffer, 1932b), he reported on the incidence of this antigen, B, and anti-B in pigs and stated that there might be a system in pigs analagous to the ABO one in humans. In just over 17% of the 385 animals examined, anti-B was present. There were, however, some anomalous results in his work, viz. the occurrence of an antigen and its corresponding antibody in 7.53% of the animals examined, and perhaps this was due to the detection of more than one antibody in these serum samples. Nevertheless it was definitely demonstrated from his work that antibodies other than anti-A were present but unfortunately no history of the animals was available as blood samples were taken from those passing through a packing plant.

In 1936, Szymanowski and Frendzel again reported that O (i.e. non-A) pig red cells possessed antigenic properties as they found that pigs vaccinated against swine fever occasionally had antibodies against A negative cells, a fact which the same group had published ten years previously, and again hinted that individual antigenic differences in porcine red cells could be shown by means of iso-immunisation.

Little interest was stimulated in pig blood groups during the next ten to fifteen years. Meanwhile, however, the pathogenesis of haemolytic disease of the newborn in humans (erythroblastosis foetalis), mules and horses was being clarified (Levine, Newark, Burnham, Englewood, Katzin, Newark and Vogel, 1941; Caroli and Bessis, 1947; and Bruner, Hull, Edwards and Doll, 1948). This stimulated Bruner, Brown, Hull and Kinkaid (1949) to investigate the possibility of haemolytic disease of the newborn occurring in piglets. They immunised 3 sows with citrated blood from the boar to which they had been mated. All the sows developed both haemagglutinins and haemolysins and gave birth to 21 piglets, all of whom had normal erythrocyte counts. However, all the piglets died within 42 hours of birth showing extreme anaemia and little icterus. They presumed that the erythrocytes of all the piglets were similar to the boar and all must have inherited the factor or factors from him. From this work they concluded that blood group factors could cause losses in baby pigs without having any evidence for the incidence in the field.

The following year, Kershaw (1950), described cases of anaemia in piglets, with deaths within the first 36 hours of life and suggested that they were similar to haemolytic disease in young foals. However, he presented no serological evidence for iso-immunisation of the dams. In 1952, Szent-Ivanyi and Szabo briefly described an outbreak of haemolytic icterus in newborn piglets in Hungary and compared it to Rhesus incompatibility in humans. They followed this up with a more detailed account of the condition in 4 litters (Szent-Ivanyi and Szabo, 1953) and presented the first serological evidence consistent with a diagnosis of haemolytic disease of the newborn. At the same time and quite independently the condition was confirmed in England (Buxton and Brooksbank, 1953a and b). Litters of jaundiced piglets dying during the first 3 days of life were seen and the condition was ascribed to blood group incompatibility. The direct Coombs test was used for diagnosis on the piglets red cells and the dam's serum was found to contain antibodies against the sire's red cells. Thus the scene was set for widespread investigations into the pathogenesis of haemolytic disease of newborn piglets with concurrent elucidation of the blood groups of pigs. Soon the condition was reported in several other countries as follows: America (Doll and Brown, 1954; Newberne, Robinson and Rising-Moore, 1956; and Quinn, 1961), Roumania (Sirbu and Paunescu, 1960), Czechoslovakia (Trunkat and Cernohlavek, 1962), Yugoslavia (Bohm, Senk and Langus, 1962; Bohm and Senk, 1966), Brazil (Martins Ferreira Neto and Lamas da Silva,

1964) and Japan (Mogi, Hosoda and Himeno, 1966).

Subsequent studies by Szent-Ivanyi and Szabo (1954) revealed that four red cell antibodies were present in the sera of the dams of their cases. These they designated anti-A, anti-B, anti-C and anti-D having identified them by the checkerboard method of absorption of antibodies from the serum by red cells from several animals in turn and subsequently back testing each absorbed sample with red cells from the same panel of animals. Anti-A was found to correspond to human anti-A and presumably corresponded to the present day anti-A which is commonly found in Ao pigs without any apparent antigenic stimulus. Unfortunately anti-B, anti-C and anti-D were never available for classification into present day nomenclature for pig blood groups. They only used a direct agglutinating technique and presumably some so-called "incomplete" antibodies may have been present but not detected. Anti-A and anti-B were found at maximum titres of 1:100 while anti-C and anti-D were generally found at dilutions of around 1:10 but never exceeding 1:20.

Meanwhile the British workers were continuing their studies. Buxton, Brooksbank and Coombs (1955), using the direct antiglobulin (Coombs) sensitisation test showed that piglets red cells were not sensitised (i.e. coated with antibody) at birth but became so after ingesting colostrum, even if this was delayed for 24 hours. Four sows were examined by them and in each case they demonstrated antibodies to the sire's red cells in the serum

of these sows. Both the direct agglutination (D.A.) and indirect sensitisation (Coombs) tests (I.S.T.) were used with titres ranging from 1:8 to 1:512 by the former technique and 1:128 to 1:640 by the latter. Hence they showed the benefit of the indirect sensitisation technique for the detection of red cell antibodies in these cases and in one of the four animals the antibodies were primarily detected by this method, i.e. they were incomplete antibodies or ones which could not bring about agglutination of red cells by themselves. Further communications (Goodwin, Hayward, Heard and Roberts, 1955; Goodwin, Heard, Hayward and Roberts, 1956) described the clinical condition in detail. Four litters of newborn piglets suffering from haemolytic disease of four different grades of severity were observed clinically (Table III). Pallor of the skin and mild jaundice were evident in some cases but the activity of the piglets was not reduced except in the terminal stages of fatal cases. In mild cases there were no clinical symptoms and the disease could be detected only by demonstrating a fall in haemoglobin in the blood and sensitisation of the red cells as shown by the direct antiglobulin test. The severity of the condition did not appear to correlate absolutely with the minimum haemoglobin (Hb) levels and newborn piglets could remain active at 3g% or less. Death seemed to be associated with the speed of fall of haemoglobin levels and it seemed that a piglet would survive provided its haemoglobin did

TABLE III

Sow	Serum antibody titre DA	Serum antibody titre IST	Severity of symptoms in piglets	Hb levels g.%	Age
A	16	64	mildest	6.3 - 9.2	5d
B	16	256	mild	5.8 - 8.8	2d
				5.9 -11.8	3d
C	64	2048	most severe (affected died)	2.6 - 6.5	28h
D	2048	2048	severe but all survived	3.8 - 5.1	3d
				3.3 - 5.3	4d

Compiled from the results of Goodwin, Heard, Hayward and Roberts (1956).

not fall below 3g% by 72 hours after birth. The presence of icterus also seemed to depend on the rate of haemolysis of the red blood cells in the piglets. If severe haemolysis took place quickly, the piglets would die before jaundice could appear. This would account for the conflicting evidence in the literature for the presence of this symptom in affected piglets and also might lead to severe cases not being diagnosed in the field. In fact various criteria were used for the diagnosis of the condition. Goodwin and Saison (1957) stated that the existence of red cell iso-antibody in the circulation of the newborn is pathological and they judged any animal that had demonstrable circulating red cell iso-antibody to be suffering from haemolytic disease while Goodwin (1957) stated that a most useful diagnostic test in the field was the slide test. Blood from an affected piglet would in

most cases agglutinate spontaneously when placed on a microscope slide. However, a more specific test is the direct antiglobulin sensitisation test which demonstrates absorption of antibody by the piglet and uptake onto its red cells.

As the clinical condition was being illuminated so the pathogenesis of the disease was attracting attention. The main question to solve was how the sows were becoming immunised by porcine red cells. As far back as 1926 Szymanowski and Wachlerowna had reported the occurrence of antibodies to A negative pig red cells in the sera of animals inoculated with crystal violet vaccine for swine fever which was prepared from the whole blood of pigs. With the increasing use of this vaccine in pigs, this was an obvious source and it was on this aspect that Goodwin and his colleagues concentrated. The recognition of the disease syndrome in Britain had coincided with increasing and widespread use of crystal violet swine fever vaccine from 1950 onwards (Roberts, 1957).

Goodwin, Saison and Coombs (1955) showed that injections of crystal violet swine fever vaccine into young pigs stimulated the production of red cell iso-antibodies. After repeated injections the titre of these antibodies in some pigs reached a high level and, within the pig population, these animals with detectable levels of serum iso-antibodies were confined almost exclusively to vaccinated herds. In a total of 147 unvaccinated pigs

they found iso-antibodies in 8 while in 152 vaccinated animals, iso-antibodies were present in 102. The highest titre in the former was 1:8 while titres of 1:512 or greater were common in the latter, especially in those vaccinated twice or more. Thus the part played by crystal violet vaccination for swine fever in the pathogenesis of haemolytic disease of the newborn piglet was established. In a subsequent publication (Goodwin and Saison, 1956) it was shown that iso-antibodies were more common among Essex and Wessex sows than Large White ones and it was found that haemolytic disease was more common in litters of the former than those of the latter. At this time sows of the Essex and Wessex breeds were popular breeding animals especially for crossing with Large White boars. This, added to the fact that crystal violet swine fever vaccine was prepared in Large White or Large White cross pigs, set up the ideal situation for outbreaks of haemolytic disease. This also demonstrated breed differences in the frequency of red cell antigens at least between the Essex and Wessex breeds on the one hand and the Large White breed on the other.

Since this work was reported, most cases of haemolytic disease of newborn piglets have been attributed to vaccination with crystal violet swine fever vaccine. It was contended that all the British cases were from sows which had been vaccinated as were those of Newberne, Robinson and Rising-Moore (1956), Bohm and Senk (1966) and

Mogi, Hosoda and Himeno (1966). In most of the other reports of the condition quoted previously no suggestion was offered as to how the iso-immunisation of the sows had taken place. However, the Hungarian workers were adamant that, while they had seen haemolytic disease in the offspring of sows vaccinated with crystal violet swine fever vaccine, they had also seen the condition in litters of sows which had received no inoculations with blood or blood products (Szabo, Szent-Ivanyi and Szeky, 1956). This has since been confirmed by Szent-Ivanyi (1970). They postulated that the sows became immunised by red cell antigens of the foetus in utero. Similarly Quinn (1961), in a short review of the condition in newborn piglets, suggested that maternal sensitisation by foetal red cells might occur but offered no evidence to support this statement while more recently, in Brazil, Martins Ferreira Neto and Lamas da Silva (1964) described haemolytic disease in the litters of two sows, neither of whom had been vaccinated with crystal violet swine fever vaccine. It was also significant that Goodwin, Saison and Coombs (1955) did find iso-antibodies in a small number of unvaccinated sows and they did not preclude the existence of naturally occurring haemolytic disease of pigs by transplacental sensitisation.

Once the Cambridge workers had established the association between crystal violet vaccination and haemolytic disease of the newborn piglet, they went on to study the epidemiology of the condition in the field and attempted to

characterise the red cell antigens and antibodies involved. They had already shown that the natural antibody, anti-A, was not concerned (Goodwin and Coombs, 1956) as the A antigen was absent from the red cells of newborn group A piglets but appeared in increasing strength at varying times during the first month of life, i.e. after the neonatal period and not until well after the time when maternally derived antibodies were absorbed from the colostrum. They then went on to study red cell antigens other than the A-O system (Joysey, Goodwin and Coombs, 1959a), and, using the sera from six sows that affected their litters with haemolytic disease, analysed 11 specific testing reagents. With these reagents 11 red cell antigens of the pig were characterized and the iso-antibodies encountered in 20 cases of haemolytic disease were enumerated. Of these the three most common were anti-3, anti-6 and anti-8; in present day nomenclature for blood groups in pigs these became anti-Lg, anti-Gb and anti-Fa respectively (Lang, 1969). However, the part played by two of these became doubtful when the frequencies of these factors in the various breeds were worked out (Joysey, Goodwin and Coombs, 1959b). The red cell type, Fa(8), was not found in Large White pigs, a fact which has since been confirmed by Gavalier, Hojny, Hradecky, Linhart and Schroffel (1966). Therefore the classically projected cases of Essex or Wessex sows being injected with swine fever vaccine containing red cell antigens from Large White pigs and subsequently being mated

by Large White boars were not going to produce haemolytic disease due to anti-Fa(8). Further of the 20 cases analysed by Joysey, Goodwin and Coombs (1959a), 9 were served by boars which were Fa positive and were thus highly unlikely to be of the Large White breed (in this communication no breeds were mentioned). Presumably anti-Fa could cause haemolytic disease when breeds of boars other than Large White were used.

Antigen 3 (Lg) was found to be of high frequency in the three main breeds studied (Essex, Wessex and Large White) and was thus unlikely to be of high significance in the production of haemolytic disease in litters of matings between these breeds. However, the third antigen, 6 (Gb), was of such a frequency as to suggest that it could be of significance as it was of high frequency in Large Whites and low frequency in Essex and Wessex pigs. Thus the chances of anti-Gb being present in the sera of Essex and Wessex pigs after vaccination with crystal violet vaccine were high and also the chances of the Large White boars to which these were mated of having the antigen on their red cells were also high.

As well as these three antibodies, several others were found, some of which were present at high titres and could have been responsible for haemolytic disease, but were undesignated at this time. Lang (1969) has given a full list of the antigens determined using the Cambridge sera and has translated them into present day nomenclature as follows:-

Present day. A Da Ea Eb Ef Fa Gb Ha Ka Ka_1 Kd La Ld Lg

Cambridge A 7 2 1 13 8 6 14 5 4&5 12 10 11 3

Antibodies to these factors were all obtained from sows which had affected their litters with haemolytic disease and, with the exception of anti-A, all could presumably play some part in the condition, either singly or in combination with others.

The only other attempt to implicate a specific blood group antigen-antibody system in the production of haemolytic disease of the newborn was by Andresen and Baker (1963), subsequently reported in a second litter from the same parents (Andresen, Preston, Ramsey and Baker, 1965). They obtained a crossbred sow which had affected three consecutive litters with haemolytic disease and in whose serum a strongly reacting anti-Ba along with weakly reacting anti-A and anti-Ea had been isolated. The vaccination history of the animal was unknown. This sow was mated to a heterozygous Ba positive boar which was Ea negative and, three weeks before farrowing, she was injected with red cells from the boar in order to boost the anti-Ba titre. The Ba positive piglets born were affected with haemolytic disease after ingesting their dam's colostrum while the Ba negative ones weren't and it was concluded that anti-Ba could be responsible for haemolytic disease of the newborn. A similar response was noted in the second litter.

To date all cases of haemolytic disease in newborn piglets reported have therefore been artificially induced

either by crystal violet vaccination or by direct injections of blood or other blood products into the dams except for a large proportion of the cases in Hungary where this mode of production has been categorically denied. However, there have been several reports of red cell antibodies occurring in the sera of sows without apparant artificial stimulation dating back to Goodwin, Saison and Coombs, 1955. Andresen and Wroblewski (1961) reported finding anti-Ka in an immune serum which could not have been produced against donor erythrocytes and they state that several examples of naturally occurring Ka antibodies have been encountered in the sera of Polish pigs. Similarly Hojny and Hala (1964) found anti-Ka in the serum of a sow which had not been vaccinated with Ka positive red cells but had previously been mated to a Ka positive boar and, in a previous communication (Hala and Hojny, 1962), they state that, in the serum of pigs which had not been immunised, they found anti-A, anti-Eb, anti-Ea, anti-Ka, anti-Gb and anti-D1.

There are thus several sporadic reports of the occurrence of red cell iso-antibodies in pigs without apparent artificial stimulation, especially in sows. The widespread use of crystal violet swine fever vaccine in the pig population throughout the world has continually confused the picture but, since this procedure was abandoned in this country in 1964 the time is now opportune to investigate the possibility of red cell antibodies other

than anti-A occurring in pigs without injections of blood or other tissues of porcine origin.

b) Iso-antibodies to porcine thrombocyte antigens

Interest in iso-antibodies to thrombocyte antigens was initiated in 1960 in Norway by the investigation of a disease of piglets characterised by thrombocytopenia and purpura (Stormorken, Svenkerud, Slagsvold, Lie and Lundevall, 1963). Piglets were born healthy but became unthrifty within one to two days and most of the litter studied died due to a haemorrhagic anaemia within a week of birth.

Previously deaths in pigs associated with purpura had been reported (Steffens, 1948; Priouzeau, 1951; and Gordon and Luke, 1952), but no thrombocyte counts had been carried out on any of the affected animals and the aetiology of these cases remained obscure. Of all these, the case reported by Priouzeau was most similar to the Norwegian one in that purpura occurred in three successive litters of the same sow and piglets were affected in the neonatal period.

Using the thrombo-agglutination technique of Lundevall (1958), the Norwegian workers showed that the thrombocytes of affected piglets were agglutinated by the dam's serum and they put forward the theory that the condition was due to maternal iso-immunisation by the foetal thrombocytes. The sow had been mated four times in succession to the same boar, the first two litters having been normal and both subsequent

ones having been affected. They believed that the antibody level in the sow could have built up with successive pregnancies in which piglets whose platelets were of a different antigenic type from her own were carried. Piglets then became affected after ingesting the antibodies in the colostrum.

At autopsy, the characteristic findings in affected piglets were paleness of various tissues, and multiple ecchymotic and petechial haemorrhages throughout the skin, subcutaneous and muscular tissues and most of the organs in the body (Nordstoga, 1965). Most of the lymph nodes were enlarged and haemorrhagic.

Subsequently a similar syndrome was reported in Britain (Saunders, Kinch and Imlah, 1966) and in Sweden (Thorne and Hakanson, 1967). In the former the thrombocytes of the sire and of the affected piglets were agglutinated by the serum of the dam but no serology was carried out in the latter case.

Using the thrombo-agglutination technique, Lie (1962 and 1966) studied the reactions of the serum from six sows which had had litters affected with thrombocytopenic purpura in Norway. In addition a seventh platelet-agglutinating serum obtained from a pig which had been immunised with citrated whole blood from another pig was studied. All the sera except one were tested with platelets from three hundred pigs; the seventh was tested with platelets from only one hundred and thirty pigs as it was in short supply.

Lie considered that four different antibodies were present and called them anti-A, -B, -C and -D. Three of the sera had anti-A, two had anti-B and the remaining two had anti-C and anti-D respectively. The report is rather brief and it is alleged that, when the different sera were absorbed with positive and negative platelets, the positive ones removed the antibody from the respective sera while the negative ones left the sera unaffected. In the light of what is known about the complexities of red cell iso-antibody/antigen systems and the difficulties encountered in obtaining reagents (i.e. antisera) which contain only one iso-antibody, it would seem that either the platelet systems are much simpler or else she was very lucky in getting seven antisera each of which contained only one antibody.

Hence it can be seen that all the work on thrombocyte iso-antibodies in pigs revolves around the syndrome known as thrombocytopenic purpura and the serological reactions of porcine platelets have only been detected by the thrombo-agglutination technique.

On the other hand, human platelet iso-antibodies and iso-antigens have been extensively studied using a wide variety of techniques. The most reliable of these have been found to be the antiglobulin-consumption, agglutination and complement fixation techniques, all of which have been found to detect platelet iso-antigens and iso-antibodies with a reasonable degree of accuracy and repeatability.

The antiglobulin consumption test was introduced by Moulinier (1955) and Steffen (1955). Platelets were incubated with platelet antibody, washed thoroughly, incubated with anti-human globulin, spun down and the consumption of anti-globulin then estimated by testing the ability of the supernatant to agglutinate sensitised human red cells as compared to the agglutinating capacity of a control sample of the same antiglobulin serum. It has been used in the investigation of iso-immune neonatal purpura in human babies (Moulinier, 1958; Goldsmith, Jenkins, Mucklow and Normand, 1965) but has been found to be too time-consuming for use in routine typing. This technique has also been investigated and described by Dausset and Colombani (1964) and Dacie and Lewis (1966).

Thrombo-agglutination is the simplest technique but is also one which has given much trouble due to the natural tendency of platelets to aggregate. Basically platelets are incubated for some time with platelet antibody and the resulting agglutination observed under a microscope. Lundevall (1958) studied many of the factors affecting the results of this test and it was on his method that Lie (1962 and 1966) based her technique.

The complement fixation technique has been used to detect a wide variety of antigen/antibody reactions (Mayer, 1961). This method was introduced into platelet immunology by Achroyd (1951) during investigations on drug-induced purpuras. Later it was used by Shulman, Aster, Leitner and Hiller (1960 and 1961) to detect platelet iso-

antigens and iso-antibodies, and subsequently modified by Aster, Cooper and Singer (1964) to a simpler qualitative test. A modification has also been used by Millot (1966) to detect thrombocyte iso-antigenic groups in cattle.

Therefore with regard to iso-antibodies to porcine thrombocyte antigens, the first priority must be to find a suitable technique for their detection. As the anti-globulin consumption, thrombo-agglutination and complement fixation techniques have been found to be most reliable in the detection of similar systems for human platelets, they would seem to be the ones to be examined at first.

Further studies are required to confirm that the thrombocytopenic purpura reported in this country is similar to that described in Norway, and to clarify the aetiology and pathogenesis of this condition. If the hypothesis presented by the Norwegian workers is correct, it should be possible to reproduce the syndrome experimentally and to find similarities to haemolytic anaemia of newborn piglets.

MATERIALS AND METHODS

1. Terminology

The traditional immunological terms used in blood group serology have been employed in the text as follows:

Antigen. A substance that elicits a specific immune response when introduced into the tissues of an animal. A hetero-antigen is one which is derived from one species and is capable of stimulating an immune response in another species while an iso-antigen is one carried by an individual and capable of eliciting an immune response in genetically different individuals of the same species but not in the individual bearing it.

Antibody. A protein produced in an individual in response to an antigen lacking in that individual and capable of combining with that same antigen. Similarly there is hetero-antibody and iso-antibody produced in response to hetero-antigen and iso-antigen respectively. A natural antibody is one which is present in the serum of a normal individual not known to have been produced in response to the relevant antigen, e.g. the anti-A and anti-B antibodies is the ABO blood group system in humans and anti-A in the A blood group system of pigs. A complete antibody is one which can coat red cells and is capable of linking them together (i.e. to bring about agglutination) while an incomplete antibody is one which can coat red cells but does not link them together (i.e. it does not agglutinate them on its own.).

Serology. The study of antigen-antibody reactions in vitro, the antibody being present in or originating from serum.

Immunisation. The administration of an antigen from one individual (the donor) into another (the recipient) to elicit an immune response and the production of antibodies to that antigen in the latter. Similarly there is hetero-immunisation and iso-immunisation as more specific terms. The latter is used to refer to either the immunisation of a mother by foetal antigens or the immunisation of an individual by the parenteral introduction of antigens from a genetically different member of the same species.

Sensitisation. This is used, firstly, for the administration of an antigen to provoke an immune response so that, on a later challenge with the same antigen a more vigorous response will ensue and, secondly, to describe the coating of cells with antibody as in the indirect sensitisation test. A sensitised animal is therefore one which has had a previous exposure to the same antigen while sensitised cells are those that have become coated with antibody.

Antiserum. Serum from any animal which contains antibodies to a stated antigen. A blood typing reagent is an antiserum by means of which it is possible to detect the presence or absence of antigens on red cells while an antiglobulin serum is an antiserum produced against the globulin fraction of serum of individuals of one species in those of another species.

Blood cellular antigenic factors. For the purpose of this text, these relate to the iso-antigens carried by the

circulating red cells and thrombocytes and are also referred to as red cell antigens and thrombocyte antigens.

2. Collection of Blood Samples

(a) Adult pigs. These were restrained by means of a nose twitch and the pressure in the ear veins raised by placing an elastic band round the base of the ear. Blood was withdrawn from a marginal auricular vein through a 20 SWG needle under aseptic conditions using the Vacutainer* System or through sterile open needles (1½ in. 16 S.W.G.), with a piece of translucent P.V.C. tubing (1½-2 in. long) attached, by gravity flow into open vials.

Where larger volumes of blood were required, as for serum or cells for absorptions of antibodies, samples were taken from the anterior vena cava of pigs in the standing position, using a 4 in. 15-16 S.W.G. needle connected to one pint sterile bottles (either dry or containing 120 ml. of acid - citrate - dextrose solution as recommended by Loutit and Mollison, 1943) by means of sterile P.V.C. tubing. To aid the flow of blood a vacuum (10-25 mm. of mercury) was applied to the system via an empty bottle which acted as a trap. The needle was inserted at a point about 1 in. anterior to and 1 in. lateral to the manubrium of the sternum on the right side, the tip being directed upwards, inwards and backwards (with respect to the pig) until the vessel was located and a free flow of blood resulted.

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* Becton, Dickinson and Company, Colombus, Nebraska, U.S.A.

(b) Piglets. All blood samples from piglets were taken from the anterior vena cava with the animal in dorsal recumbency. The point of entry of the needle was in the right supraclavicular fossa on a line from the manubrium of the sternum to the base of the ear, about ½ to 1 inch, anterior to the manubrium, the point of the needle being directed forwards, downwards and inwards (with respect to the operator) between the first pair of ribs. 5 ml disposable syringes with 1½ inch 20 SWG disposable needles were used, slight negative pressure being applied to denote when the needles entered the blood vessel. 2-3 mls of blood were withdrawn at a time and immediately dispensed into bijous for haematology and serology.

For thrombocyte counts great care had to be exercised in taking the blood samples to ensure that the clotting mechanism was not initiated and, in the case of thrombocytopenic animals, that the vessels used could be easily cauterised to prevent haemorrhage afterwards. Therefore, samples were taken from pricks in the marginal auricular veins directly into platelet diluting fluid in white cell pipettes with minimum delay. The skin over the vein was wiped with dry cotton wool unless the ear was dirty in which case surgical spirit was used, adequate time being allowed for it to dry before taking the sample. Where required a tourniquet in the form of a fine elastic band was placed over the base of the ear - provided the piglets were kept in a warm creep before sampling this was not usually necessary.

After sampling, a dry piece of cotton wool was placed on the wound and bleeding stopped quite quickly in normal piglets. In thrombocytopenic ones, if the blood was still oozing out ten minutes after sampling, mild cautery was used to aid haemostasis.

(c) Rabbits. Blood samples from rabbits were obtained from the marginal auricular veins. The skin over the vein was shaved and wiped with surgical spirit and the tip of the ear moistened with xylol to produce vaso-dilatation. The vein was then nicked longitudinally with a scalpel blade and the blood allowed to flow over petroleum jelly into 5 ml bijous or 1 oz wide-necked vials containing A.C.D. (for cells) or dry (for serum). Up to 100 ml of blood could be collected from a rabbit by this method provided it was not disturbed by extraneous noises. After bleeding, the xylol was washed off and haemostasis aided by placing a piece of dry cotton wool over the punctured blood vessel.

3. Blood Sampling Routine

(a) Sows. For the monitoring of anti red cell antibody levels in serum, blood samples were collected from sows at fortnightly intervals during pregnancy, at parturition, at five and ten days post partum and then at weekly intervals up to weaning.

(b) Piglets. At birth all piglets were immediately removed from their dams to prevent them from ingesting

colostrum and all were blood sampled and identified by a system of ear notches before returning. All members of a litter were returned to their dam simultaneously in order that each piglet might have an equal share of and have access to the colostrum at the same time.

In those litters in which red cell investigations were carried out, further blood samples were taken at 24 - 36 h, 4 d, 7 d, 13 d and 20 d later. In two litters (from sows 301 and 302, see later) weekly samples were taken after this up to 20 w of age.

Similarly, in those litters in which thrombocytes were studied, further platelet counts were carried out 12 h and 36 h post colostrum, subsequently at intervals of 2 d up to 3 w of age and then twice weekly until 5 w old when observations ceased.

4. Methods used for the Detection of Red Cell Antigen/ Antibody Reactions.

(a) Red Cell Suspensions. Whole blood from adult pigs was collected in 7 ml vacutainer tubes containing 1 ml acid-citrate-dextrose (A.C.D.) solution B (Becton, Dickinson & Company) and erythrocytes were either used fresh or having been stored at 4°C for a maximum period of one week. For red cell typing of the piglets, precolostral blood samples were dispensed into bijous containing the A.C.D. solution recommended by Loutit and Mollison (1943).

For test purposes red cells were washed four times and resuspended in phosphate buffered saline (0.9% sodium

chloride w/v aq. dist. with 10 ml of 0.2M phosphate buffer pH 7.2 added per litre) in 75 x 10 mm tubes to a concentration of either 1.5% or 2% according to the technique to be used. Suspensions were standardised using an Eel Colorimeter with a red filter, No.608.

(b) Serum samples. Serum was obtained by collecting blood in dry 10 ml Vacutainer tubes (adults) or dry bijous (piglets). These were placed in an incubator at 37°C for one hour to promote clot formation and left in a refrigerator at 4°C overnight to allow for adsorption on to the red cells of any cold auto-antibodies present in the serum as well as giving time for adequate clot retraction to take place. Serum was decanted the following day and any red cells present removed by centrifugation before inactivating the complement by heating in a water bath at 56°C for half an hour. Serum samples were then stored at -20°C and tested for red cell antibodies in batches in order to keep the test conditions as standard as possible. Serum dilutions in saline were prepared in 50 x 10 mm tubes.

(c) Serological techniques. Erythrocyte antigen-antibody reactions were detected by means of the three standard techniques used routinely for pig red blood cell typing in the Blood Group Research Unit. All tests were carried out in 50 x 7 mm tubes using a drop technique, i.e. 1 drop of cell suspension to 1 drop of serum dilution, with pasteur pipettes calibrated to drop approximately 0.025 ml each time. For the red cell typing, in order to

economise on reagents, small drops (approx. 0.015 ml) were used in the direct agglutination and haemolytic techniques.

(i) Direct agglutination test. One drop of a 1.5% suspension of red cells in phosphate buffered saline was added to one drop of serially doubling dilutions of serum and incubated at 37°C for one and a half hours. Reactions were read on clean microscope slides at 100 x magnification and scored descriptively and/or numerically as follows:-

Percentage cells agglutinated	Description	Score
100	V	5
80	++	4
60	+(+)	3
40	+	2
20	(+)	1
<20	W	0

50% end point titres of antibodies in the serum were calculated algebraically from the formula $\frac{A-B}{(A-B) + (D-C)}$ according to Lauffer, Carnelly and McDonald (1948), and expressed as the $\log_2$ of the reciprocals of the dilutions at these points. For example, consider the hypothetical reaction between cells from pig X and serum from pig Y where the 50% end point occurs between dilutions 1:2 and 1:4 :-

Cell Source X; Serum ex Y at	1:1	1:2	1:4	1:8	1:16	1:32
Description of reaction	V	++	+	(+)	-	-

Positive Score	5	4	2	1	0	0
Negative Score	0	1	3	4	5	5
Accumulated Positive Scores<-	12	7(A)	3(C)	1	0	0
Accumulated Negative Scores->	0	1(B)	4(D)	8	13	18

Therefore the $\log_2$ of the reciprocal of 50% end point titre of the antibodies to cells ex X in serum ex Y is

$$2 + \frac{7-1}{(7-1) + (4-3)}$$, i.e. 2.86.

(ii) Indirect sensitisation (Coombs) test. (Coombs, Mourant and Race, 1945). One drop of a 2% suspension of red cells in saline was added to one drop of serially doubling dilutions of serum and incubated at 37°C for one and a half hours as in the direct agglutination test. The excess serum was then removed by washing the cells four times in phosphate buffered saline before adding one drop of a 1 in 50 dilution of rabbit anti pig globulin serum. After incubation for a further hour at 37°C, the reactions were read macroscopically over a concave mirror and recorded visually or numerically according to the sedimentation patterns of the cells in the tubes. In this case arbitrary scales of ++, +(+), +, (+) and 4-1 were used according to decreasing strength of reaction and they were not comparable with the scales used for the direct agglutination test. 50% end points were calculated as for the direct agglutination technique and titres expressed as the $\log_2$ of the reciprocal of the dilution at that point.

(iii) Haemolytic technique. This test was carried out according to Imlah (1964). One drop of a 1.5%

suspension of red cells was added to one drop of serially doubling dilutions of serum and left at room temperature for 30 minutes after mixing. One drop of fresh rabbit complement previously absorbed at 4°C for 30 minutes with washed packed A pig cells (1 vol. of packed cells to 4 vols. of serum) was then added and the tubes were placed in an incubator at 20°C and mechanically shaken for a minute every hour for six hours. Readings were recorded macroscopically according to the degree of haemolysis of the red cells on a numerical scale 0-5 which corresponded to a range of haemolysis 10-100% (Imlah, 1964). Smaller degrees of haemolysis were recorded as trace (tr.).

With this test two negative controls were run with each batch of tests. The first tube omitted antiserum but contained one drop of complement, one drop of saline and one drop of cells and was designed to test for the removal of natural (anti-A) and species antibodies within the complement. The other tube contained two drops of saline and one drop of cells and this acted as a control on the tonicity of the red cell membranes to withstand the test.

(d) Routine Red Cell Typing. All animals studied were typed for red cell factors by the reagents used routinely in the Blood Group Research Unit and the three techniques described. At first 19 factors were covered but this was increased to 29 by the end of the investigation, 6 new reagents having been produced in the laboratory in that time.

Typing of piglets was carried out on the precolostral

blood samples, dispensed into bijous containing ACD, in order that the specificity of the reagents might not be masked by maternal red cell antibodies absorbed from the colostrum.

In 1969 a panel of forty pigs, including some of the animals used in these experiments, were typed for 54 factors by J. Hojny, Libechov, Czechoslovakia. Where available, the full typing from this is quoted.

(e) Panel of animals of known red cell type. For the detection of red cell antibodies, a panel of animals of known blood type was selected as donors of red cells in order to cover all the known red cell factors - at first this was the range covered by the Blood Group Research Unit reagents, but, once the Czechoslovakian results were available, animals were selected from the forty fully typed ones.

Most of the testing was done with red cells from five animals carefully selected to make recognition of antibodies easy when they were present. Unfortunately this panel could not be kept exactly the same throughout the whole period of the investigation due to wastage by illness and death.

All the animals used for antibody detection with their blood types as far as they were known at the time of use were as shown in Table I. Similarly for continuous screening of antibody levels, a panel of 5-6 animals was chosen from this list and also included the boar to which the sows had been mated where possible.

Table 1.

Red Cell types of Animals Used for Antibody screening (1-16) and Experimental animals (8, 10 and 17-24)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Gb/Sc	
	a o	a b	a b	a b	a b d e f g h j k l	a b	a b	a b c d	a b	a b	a b c d e	a b c d f g h i j k l	a c d g	a b	a b	9 16 19 21	
1. W3184	+ .	+ .	. /	. /	+ . . + . + / / / /	. +	+ .	. + / /	+ .	. /	. + / . /	+ + + / / + / / / / /	/ / / /	/ /	/ /	+ . / /	W3184
2. PY4731	+ .	+ .	. /	. /	. . + + + + + / / / /	. +	. +	+ . / /	+ .	. /	. + / . /	. + + / / + / / / / /	/ / / /	/ /	/ /	+ . . /	PY4731
3. T38P12	. +	+ .	. /	. /	+ + + + + + / / / /	+ .	. +	+ . / /	+ +	+ /	+ + / . /	+ + . / / . / / / /	. / / /	/ /	/ /	+ . . /	T38P12
4. P20	. +	+ .	. /	+ /	. + + + . + / / / /	. +	. +	. + / /	/ .	. /	+ + / + /	+ + + / / + / / / / /	/ / / /	/ /	/ /	+ . . /	P20
5. RW	. .	+ .	. /	+ /	. + + + . + + . + /	+ +	+ .	+ . + /	+ +	. /	+ + + . +	+ + + + . + + + . . .	. . . /	. +	. +	+ . . /	RW
6. 6	. +	+ .	. /	+ /	. + + + . + + . + /	. +	. +	. . + /	. /	. /	+ + + . +	. + + . . + . + . . .	. + + /	+ +	. +	+ . + /	6
7. 8544	+ .	+ .	. /	. +	+ . + + . + + . + +	. +	+ +	. . . .	+ .	. .	. + . . .	. + . + + . . + . . .	+ . . .	. +	. +	+ . . .	8544
8. 311	+ .	/ /	/ /	. /	. . + + + + + . . /	. +	+ +	. . + /	+ .	+ /	. + . . .	+ . + + . + + + + . +	. . . /	+ +	. +	+ . . .	311
9. 308	. +	+ .	. /	+ +	+ . + + + + + . + +	. +	+ +	. . + +	+ .	+ .	+ + + . +	+ + + . . + + + + . +	. . . .	+ +	. +	+ . . .	308
10. 8	. +	/ /	/ /	+ /	+ . + + . + / / / /	. +	. +	/ . / /	/ .	/ /	+ + + . .	+ / / / / + / / / / /	/ / / /	/ /	/ /	+ . . .	8
11. BWR	. +	+ .	. /	+ +	. + + . . + . . + .	+ .	+ +	+ . + +	+ .	. .	. + . . .	. + . + + + . + . . .	. . . .	. +	. +	. . . .	BWR
12. L412	. +	+ .	. /	. +	. . + + + . + . + .	. +	. +	. . . .	+ +	+ .	+ . + . .	. + + . . + . + . . .	. + + .	. +	. +	+ . . .	L412
13. 191	. +	+ .	. /	. +	. . + + . + + . + .	. +	+ +	. + + +	+ .	+ .	+ + . + +	+ + . + + . + + . . .	. + + .	+ +	. +	+ . . .	191
14. L413	. +	+ .	. /	. /	. + + + + + / / / /	. +	. +	/ / / /	/ +	+ /	. . . . /	. + + / / + / / / / /	/ / / /	/ /	/ /	+ . . /	L413
15. 71P16	+ .	+ .	. /	. /	. + + . . . / / / /	. +	. +	+ . / /	/ +	. /	+ . / . /	. + + / / + / / / / /	/ / / /	/ /	/ /	+ . . /	71P16
16. 72	+ .	+ .	. /	. /	. + + . . + / / / /	. +	+ +	. . / /	+ +	+ /	. + . / /	. + + / / + / / / / /	. / / /	/ /	/ /	+ . . /	72
17. 88	. +	/ /	. /	. /	. + + . . + / / / /	. +	. +	/ . / /	/ +	/ /	. + . . .	. / / / / + / / / / /	/ / / /	/ /	/ /	+ . . .	88
18. 405	. +	/ /	/ /	/ /	. + + + . + / / / /	. +	. +	/ . / /	/ +	/ /	. + . . .	. / / / / + / / / / /	/ / / /	/ /	/ /	+ . . .	405
19. 1347	. +	/ /	/ /	/ /	. + + + . + / / / /	. +	. +	/ . / /	/ +	/ /	. + . . .	. / / / / + / / / / /	/ / / /	/ /	/ /	+ . . .	1347
20. 1350	. +	/ /	/ /	/ /	. + + + . + / / / /	. +	. +	/ . / /	/ +	/ /	. + . . .	. / / / / + / / / / /	/ / / /	/ /	/ /	+ . . .	1350
21. BFM II	. +	/ /	/ /	. /	+ + + + . + / / / /	. /	. +	/ / / /	/ +	/ /	. + / . /	. / / / / + / / / / /	/ / / /	/ /	/ /	/ . / /	BFM II
22. 301	. +	+ .	. /	. +	. + + . . + . . + .	. +	. +	+ . . .	+ +	+ +	+ . . + +	. + + . . + . + . . .	. . . .	+ +	. +	+ . . .	301
23. 302	. +	/ .	. /	. /	. + + + . + + . + /	. +	+ +	. . . /	+ +	+ /	+ . . + +	. + + . . + . + . . .	. . . /	+ .	. +	/ . / /	302
24. 305	+ .	/ .	. /	+ /	. . + + + + + . + /	. +	+ +	. . + /	+ +	+ /	+ . + . +	. + + . . + . + . . .	. . . /	. +	. +	+ . / /	305

+ - positive . - negative / - not typed

(f) Characterisation of antibodies. Antibodies were characterised and quantitated by the usual checkerboard absorption and titration methods, using mainly the direct agglutination and indirect sensitisation techniques as described by Joysey, Goodwin and Coombs (1959a) and Lang (1969), and cells from the reference panel of animals.

For absorptions cells were washed 3 times in saline then packed in the bottom of a tube by centrifuging at 1500 G for 30 minutes. The supernatant saline was removed and an equal amount of serum added to the cells. These were mixed and placed in an incubator at 37°C for 30 minutes. After centrifuging again at 1500 G for 30 minutes the supernatant serum was carefully removed with a clean pasteur pipette and the process repeated. In most cases 2 absorptions were enough to remove the antibodies reacting with a particular cell.

In the system adopted all factors carried by the cells not reacting with the serum could be immediately eliminated and also those carried by the donor of the serum itself. Then by absorbing by the reacting cells in turn, a recognisable pattern of reactions became apparent. Secondary checkerboards and sometimes even tertiary ones had then to be planned to sort out antibodies by selecting further animals of known-blood type with the required combinations of the antigens required to separate the antibodies.

5. Methods used for the Detection of Thrombocyte Antigen/

Antibody Reactions.

(a) Isolation of Thrombocytes. The isolation and washing of thrombocytes was carried out using the technique of Lundevall (1958) with certain modifications. 20-25 ml of blood were collected by the open needles technique in wide mouthed vials (28.4 ml) containing 2½ ml 2% ethylene diamine tetracetic acid (EDTA) as anticoagulant made up as follows:-

2 Na - EDTA	20 g
Na Cl cryst.	5 g
N/1 NaOH	35 cc
aqua dist ad	1000 cc

The samples were centrifuged at a relative centrifugal force (RCF) of not more than 200 G for 12 minutes in an MSE bench swing-out centrifuge at room temperature. With most adult pigs this was sufficient to sediment almost all of the red cells. However, with the blood from younger animals the RCF had to be increased to 300 G to bring about adequate sedimentation of erythrocytes and leucocytes and, in these cases, the yield of thrombocytes was poor as many of them were also sedimented by this force.

The platelet-rich-plasma was then sucked off and spun at 600 G for another 12 minutes. In some cases this had to be increased to 800 G to bring down all the platelets. After decanting the plasma, the platelets were resuspended in isotonic saline to which had been added 1/10 volume of 2% Triton*, the resuspension being aided by the use of a

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* Triton WR - 1339 - Ruger Chemical Co. Inc., Irvington-on-Hudson, New York.

Whirlimixer*. For all techniques platelets were washed in a similar way three times in this solution before final suspension and standardisation by counting in a Neubauer platelet counting chamber (see later).

Where very pure suspensions of platelets were required as for production of antisera and in certain serological tests, the contaminated red cells were lysed by adding a small quantity of distilled water to the platelet button and suspending for 30 seconds in this before restoring isotonicity. Using this technique virtually all the red cells could be eliminated leaving the final contaminants as leucocytes at a concentration of less than 2% of the thrombocytes. This was especially important for techniques such as complement fixation and antiglobulin consumption where contaminating antigen/antibody reactions could lead to false results.

(b) Serological techniques

(i) Thrombo-Agglutination Test (Lundevall, 1958). For this test the platelet suspensions in Triton-saline were standardised to approximately 300,000/mm^3 and erythrocyte lysis was unnecessary as any contaminants did not influence the reaction.

1/10 volume of 2% EDTA was added to the antiserum before use to control non-specific aggregation of thrombocytes. One drop of serum so treated was mixed with one drop of platelet suspension within a ring of lacquer paint about 2 cm. in diameter on a microscope slide. The slides were then

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* Whirlimixer - Fisons Scientific Apparatus Ltd., Loughborough, Leicester, England.

placed in a moist chamber, and agitated by reciprocal motion in a horizontal plane at a frequency of 70 cycles per minute, and an amplitude of 1 cm for half an hour at room temperature. After an additional half hour at the same temperature, readings were made in a dark field microscope at 100 x magnification, the slides being agitated manually just before reading. The reactions were photographed using Ilford Pan-F film, to be kept as a permanent record so that comparisons between readings might be less subjective.

(ii) Fluorescent Antibody Technique. The technique recommended by K.B. Fraser and M. Haire, Queen's University, Belfast, Northern Ireland, for the conjugation of antisera with fluorochromes was followed.

Fluorescein isothiocyanate (FITC) was mixed with ten times its weight of celite powder which acted as a vector. Whole serum was then conjugated with the celite - FITC mixture (40 mg/ml) before precipitating the globulins using an equal volume of 50% saturated ammonium sulphate and washing the precipitate twice in 40% saturated ammonium sulphate. The globulin deposit was redissolved in distilled water and passed through a column of Sephadex G-75 beads, equilibrated with 0.01 M phosphate buffered saline, to remove unconjugated FITC. The conjugated protein band was readily visible and passed through the column before the unconjugated fluorochrome. Finally to cut down non-specific staining the conjugated serum was absorbed with homogenised porcine liver overnight at 4°C at

a proportion of 1 volume of serum to 3 volumes of dry liver powder.

Smears of washed platelets were air-dried, fixed with absolute alcohol and stained with the conjugate for 30 mins. before washing and mounting in glycerol for microscopical examination at 100 x magnification under ultraviolet light.

(iii) Immuno-Diffusion in Agar Gel. This was performed on microscopic slides as described by Lang (1969).

10 ml of Agar solution (1.5% Difco "Special Agar-Noble" in phosphate buffered isotonic saline, pH 7.2) was poured on to each row of three microscope slides (0.8-1.0 mm. thick) previously fixed with a glycerine/agar mixture on to plastic LKB racks which were held on a levelling table. After maturing, usually overnight, the wells were cut using the LKB Gel punch set.

Two patterns of wells were used. The first was the honeycomb pattern of 31 wells per slide as used by Lang (1969) and the second was a pattern of a central well surrounded by six similar wells, all being equidistant. All wells were 3 mm. in diameter and the diffusion distances were 2 mm. in the former and 5 mm. in the latter.

Great care was exercised to ensure that the thrombocyte suspensions were as pure as possible and erythrocytes were lysed with distilled water. After washing three times and resuspending in normal saline containing 2% EDTA the platelets were counted before disintegrating by supersonic vibrations in 1 oz. vials for

2 mins. using a Willems Polytron PT 10 apparatus.*

Reagents were applied to the wells either using pasteur pipettes or 5 μl. graduated pipettes. The latter was more time-consuming but more accurate in the amount dispensed.

48 hrs. were allowed for diffusion and precipitation to take place in moist chambers at room temperature before washing out the unprecipitated proteins with two changes of isotonic saline over 24 hrs. followed by distilled water (to remove the sodium chloride) for 6 h. The agar was then dried with the help of strips of lint-free filter paper.

Precipitin lines were demonstrated by staining with amido black (0.1% in a 50:50:10 Methanol, distilled water and acetic acid mixture which was also used as washing fluid). Slides with the agar films were immersed in the stain for 5 mins followed by four 10 min periods in washing fluid.

(iv) Complement Fixation Technique. This was carried out as recommended by Mayer (1961) with certain modifications using the following reagents:-

Diluent - Isotonic barbital buffered saline**, pH 7.2.

Antigen - For this test, the platelet suspensions had to be as pure as possible and erythrocytes were lysed with distilled water as previously described. After isolation the platelets

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* The Northern Media Supply Ltd., Hull, England.

** CFT tablets - Oxoid Ltd., London.

were counted and washed in diluent to get rid of EDTA which is anticomplementary. Standardised suspensions were then subjected to supersonic vibrations as described for immuno-diffusion so that the platelets could not aggregate. Sodium azide (1:5000) was added as a bacteriostat and the suspensions of antigens were used either fresh or after storage at 4°C.

Antiserum - Before use in this test, serum samples were treated in several ways. Some were heated in a water bath at 56°C for half an hour to inactivate the natural complement present. However, the procomplementary activity of inactivated porcine sera had to be taken into account. Either it was accepted that this phenomenon would give a prozone and positive reactions would not be apparent up to dilutions of serum above 1 in 10 or 1 in 20, or it was eliminated using formalin after Cowan (1961).

Complement. Preserved guinea pig serum* was used as a source of complement, the number of 50% haemolytic units being used varying from three to five.

Haemolytic system. The haemolytic indicator system consisted of a 2% suspension of sheep red cells sensitised with two 100% haemolytic units of rabbit haemolytic serum**. The sheep red cells used were collected in Alsevers solution (Mayer, 1961) and stored at 4°C for one week before use. Only red cells from

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* Wellcome Research Laboratories, Beckenham, England.

** Wellcome Research Laboratories, Beckenham, England.

sheep of blood type r were used to prevent reaction between them and the natural blood group antibody, anti-A, which might be present in some of the porcine sera (in the sera of pigs whose red cells are group Ao).

Equal volumes of sheep red cell suspension and diluted haemolysin were mixed and incubated at 37°C for 20 mins. before adding to the antigen/antibody/complement system.

Procedure. A four volume system was used. Initially equal volumes of serum dilution, antigen and complement, added in that order, were mixed and allowed to stand at 4°C, 20°C or 37°C for 18 h, 3 h, or 1½ h respectively. The same volume (i.e. as each previous reagent) of sensitised sheep red cells was then added, the mixture well shaken and allowed to react at 20°C for 1 h with further shaking every 15 min.

Tests were carried out either in W.H.O. persplex plates** or 50 x 7 mm. tubes. In the former 0.1 ml volumes of each reagent were used while, in the latter, a drop technique (approximately 0.025 ml. per drop) was developed to economise on reagents. The red cells were allowed to settle before readings were made by the naked eye. Where tubes were used they could be spun in a centrifuge at 300 G to enable readings to be taken more quickly.

** Messrs. Prestware Ltd., Southdown Works, Kingston Road, London, S.W. 20.

The degree of haemolysis was scored by eye on a scale 0 - 4 as follows.

4	≡	Complete haemolysis
3	≡	75% of the red cells haemolysed
2	≡	50% " " " " "
1	≡	25% " " " " "
tr	≡	10% " " " " "
0	≡	No haemolysis

(v) Antiglobulin Consumption Test. The techniques of Dausset and Colombani (1964) and Dacie and Lewis (1966) were followed and modified for this test.

Platelet suspensions were prepared as pure as possible and standardised as already described. Initially 0.5 ml of serum was added to 1 x 10^9 platelets in 1 x 7 cm. siliconised tubes, thoroughly mixed using the whirlimixer and incubated for 1 h at 37°C. New tubes were used for each test, the old ones being discarded to prevent contamination due to the difficulty experienced in washing tubes completely free of platelets and serum. Varying volumes of serum and numbers of platelets were then tested to find the optimal quantities of each for the test.

After washing ten times in isotonic saline which contained 2% EDTA, 0.1 ml of a dilution of rabbit anti pig globulin of known titre was added to the platelet button. The dilution of anti-globulin used was the eighth from the end-point in a doubling dilution titration. For example, if the end-point was 1:1024, a dilution of 1:8 was used.

After thorough mixing with the whirlimixer this was allowed to sit at room temperature for 15 min before centrifuging. Finally the antiglobulin content of the supernatant was determined by titrating with the indicator red cell system as follows:-

A modification of the indirect sensitisation test (4.c(ii)) was used. Several reagents in routine use in the Blood Group Research Unit by this method were tested for their suitability for use in the indicator system along with pig red cells carrying the respective antigen. Equal volumes of a 2% suspension of washed red cells and the recommended dilution of the reagent were mixed and incubated for 1½ h at 37°C before washing four times. This stage corresponded to the first stage of the I.S.T. The red cells were then resuspended in phosphate buffered saline to a concentration of 2% and one drop (approx. 0.025 ml) added to a similar drop of each dilution of antiglobulin. After incubating for an hour at 37°C, reactions were read macroscopically as described for the I.S.T. Controls were included for non-specific uptake of antiglobulin by the platelets themselves (i.e. platelets + saline; no serum) and for the actual antiglobulin titre each time and the test was interpreted as follows:-

Not more than one dilution drop in antiglobulin titre with the platelets alone ensured that the platelets were washed adequately for a start and that they were not taking up the antiglobulin non-specifically.

<u>Positive reaction</u> - a fall in antiglobulin

titre of at least two dilutions between that added to the platelets on which serum had been allowed to act and that added to the platelet control.

Indefinite reaction - a fall in antiglobulin titre of only one dilution between these two. Those in this category were repeated.

Negative reaction - no fall in titre between the two.

In addition a preimmunisation or negative serum was included for each platelet sample to test for non-specific uptake of serum by the platelets.

(vi) Absorption of antibodies. Anti-thrombocyte antibodies were absorbed from serum in a similar way to that used for red cell antibodies. The serum was added to washed platelet deposits in siliconised tubes and, after mixing using the whirlimixer, incubated for 30 mins at 37°C. By centrifuging at 1,000 G for 10 min, the platelets were again deposited on the bottom of the tube to allow the serum to be decanted. Hence any antibodies absorbed on to the platelets were removed from the serum. In most cases two absorptions were enough to remove the antibodies reacting with a particular platelet sample.

The first stage of the antiglobulin consumption test provided a convenient method for absorbing antibodies, the supernatant serum being retained and retested each time until no antibody activity remained.

6. Experimental Animals - Red Cell Studies.

(a) Minimal Disease Herd. In 1962 a Minimal Disease herd of pigs had been set up at the Veterinary Field Station. The original animals had been obtained by hysterectomy and complete records of all animals were available. From these it was possible to ascertain that none of the animals had been injected with blood in any form and it was decided that these were ideal animals for the anticipated experiments. Initially serum samples were taken from all the adult animals in the herd and tested for red cell antibodies by techniques already described.

(b) Animals in Routine Parentage checking. Serum samples from all adult pigs sent for routine parentage checking to the Blood Group Research Unit, under the Meat and Livestock Commission's Elite and Accreditated Herds Scheme, during the years 1968-69 and 1969-70, were examined for the presence of red cell antibodies. As attempts are now being made to increase the rate of genetic improvement, the offspring of young breeding animals are now being tested under this scheme, i.e. sows with their first or second litters. Hence it was most unlikely that any of these animals had been vaccinated with crystal violet swine fever vaccine.

All serum samples were tested with red cells from a panel of five animals, viz. 311, 308, BWR, L412 and 191, using the direct agglutination, indirect sensitisation and haemolytic techniques as already described. For the full red cell types of these animals, see Table I. Antibodies were characterised by absorption and titration as earlier

described and their incidence in sows and boars compared.

(c) Sows in the Blood Group Research Unit Piggery. From the routine screening of sows' sera (6b), two sows, 301 and 302, were obtained which had red cell iso-antibodies present in their sera. These were brought into the Blood Group Research Unit, for further studies.

(d) Dams of Thrombocytopenic Piglets. A condition in piglets known as thrombocytopenic purpura was first described in Norway (Stormorken, Svenkerud, Slagsvold, Lie and Lundevall, 1963) and subsequently in this country (Saunders, Kinch and Imlah, 1966). It is considered by these workers that the thrombocytopenia is due to iso-immunisation of the sow by incompatible foetal thrombocyte antigens. Antibodies so produced are absorbed by the piglets from the colostrum to produce thrombocytopenia in these piglets whose thrombocytes carry the incompatible antigens.

If iso-immunisation with thrombocytes was a possibility, it was anticipated that iso-immunisation to red cell factors might have taken place and could be used as an indicator system. It was decided, therefore, to examine the sera of the dams of thrombocytopenic litters to see if red cell antibodies had been produced. Serum samples from 24 dams were examined by means of the direct agglutination, haemolytic and indirect sensitisation techniques, using red cells from five animals of known red cell type in order to cover various combinations of all known types in the reference panel of the Blood Group Research Unit as already

described. Antibodies were characterised by checkerboard absorption and titration methods as already described.

(e) Piglets. To assess the effects on the piglet red cells of red cell antibodies absorbed via the colostrum, four litters were studied - these from 301, 302, 88 and 405, all sired by Balhary Field Marshall 2. This boar was selected as the sire as he was heterozygous for the factor Ea and hence Ea negative animals could be expected in the litters as well as Ea positive ones, the former to act as controls for the effect of anti-Ea on the red cells of the latter.

7. Experimental Animals - Thrombocyte Studies

(a) Dams of litters affected with thrombocytopenic purpura. Four sows which had produced litters affected with thrombocytopenic purpura were purchased along with the boars to which two of them had been mated.

(b) Survivors of thrombocytopenic litters. Four surviving piglets of a litter which had been affected with thrombocytopenia purpura were obtained for further studies.

(c) Animals used for thrombocyte hetero-immunisation.

(i) Rabbit/Pig. Two lop-eared rabbits were immunised intramuscularly with four 1 ml injections of a suspension of pig platelets containing 5 x 105 platelets per cu.mm. Injections were given twice a week for two weeks and the rabbits were bled ten days after the last injection.

(ii) Pig/Cow. A six-month old crossbred pig, No.228, was immunised with thrombocytes isolated from the

blood of an Ayrshire cow. Two intramuscular injections of an emulsion of 5 ml. Freund's complete adjuvant and 5 ml. of platelet suspension (5 x 105 platelets per cm. mm.) were given at an interval of 2 weeks. 500 ml. of blood was collected from the pig after a further two weeks.

8. The Production of Antibodies to the components of porcine sera.

Antibodies to porcine serum proteins were prepared in rabbits for use in various tests as follows:

(a) Anti-pig-globulin for the indirect sensitisation and the antiglobulin consumption tests. For these, reagents containing antibodies against all immunoglobulins were required and prepared as described by Lang (1969). Sera were titred and standardised against porcine red cells sensitised with various red cell typing reagents to find a common optimal dilution as recommended by Dunsford and Grant, (1959.)

In addition potent antisera were produced using a modification of the technique of Hamilton Fairley and Harris (1962) for the production of anti-human-globulin sera. This involved the injection of rabbits with their own cells which had been sensitised with heterophile antibodies, occurring in porcine sera.

(i) Animals. Lop-ear rabbits weighing 3-4 Kg. were used.

(ii) Preparation of sensitised rabbit cells. Rabbit blood was collected from the ear veins into acid-

citrate-dextrose anticoagulant and the cells washed 6 times in phosphate buffered isotonic saline (P.B.S.), pH 7.2, before packing by centrifuging at 1600 G for 15 mins, 2 ml. of a 1 in 2 dilution in saline of porcine serum inactivated by heating at 56°C for 30 mins. were added to 1 ml. of packed cells, thoroughly mixed and incubated at 37°C for 1 hour. After washing in PBS a further six times, the cells were resuspended in the serum to a volume of 4 ml.

(iii) Immunisation procedure. Each animal was injected slowly intravenously with its own cells prepared as above. Each course of immunisation consisted of 4 intravenous injections of freshly sensitised cells at 3 day intervals. A rest period of 10 days was allowed between each course and usually 3 such courses were required to produce a potent antiserum. Rabbits were bled 10 days after the last injection and the antisera tested and standardised as described by Lang (1969) and also by immuno-electrophoresis (Williams and Grabar, 1955).

(b) Anti-pig-serum for immuno-electrophoresis. In this technique antisera containing antibodies to a wide spectrum of porcine serum proteins were required. Consequently these were prepared by injecting rabbits with whole serum from pigs as described by Lang (1969).

9. Techniques for the Characterisation of Antibodies.

(a) Gel Filtration. Gel filtration was performed using Sephadex G.200* beads in a Pharmacia* column K.25/45

* Pharmacia, Uppsala, Sweden.

(2.5 x 45 cm) with an upward flow adaptor after Flodin and Killander (1962).

(i) Packing of the column was carried out as recommended by the manufacturers in their booklet "Sephadex - gel filtration in theory and practice", under a hydrostatic head of 12-15 cm.

(ii) Sample application. The column was loaded with not more than 6 ml of serum for each run via the flow adaptor.

(iii) Elution was performed in an upward direction with the help of a peristaltic pump at a flow rate of 10.5 ml/h using a buffer solution of 0.1 M tris/HCl, pH 8.0 in 1 M sodium chloride (Killander 1964).

(iv) Collection of eluate. The elution pattern of the proteins was monitored semiquantitatively using the absorption of ultra-violet rays at 2537Å on an LKB Unicord absorptiometer* with a quartz measuring cell, the pattern being recorded on an LKB Chopper Bar recorder at a chart speed of 10 mm/h. Fractionations gave the usual triple peak (19S, 7S and 4.5S) distribution of proteins followed by the 4th peak of small molecular weight material.

The eluate was collected in 7 ml tubes on an LKB Radirac automatic fraction collector*. With the latter set to move every 20 min, 3.5 ml was collected in each tube. The contents of consecutive tubes in the 19S and 7S peaks were pooled avoiding those where the peaks overlapped,

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* LKB - Produkter AB, Stockholm, Sweden.

and the immunoglobulins precipitated by adding half the volume of saturated ammonium sulphate (Stelos, 1967) and allowing to sit at 4°C for 15 minutes. The sediment was recovered by centrifuging at 1500 G for 15 minutes at 4°C and washed twice in 40% saturated ammonium sulphate in a similar manner before dissolving in isotonic phosphate buffered saline, pH 7.2 to approximately the volume of the serum applied to the column initially. The contents of consecutive tubes in the 4:5 S (albumin-rich) and 4th peaks were similarly pooled and concentration to the original volume was achieved by dialysis in Visking tubing against a 5-10% solution of Carbowax 4,000.

Dialysis in both cases was then performed overnight at 4°C in Visking tubing against 2 l. of isotonic phosphate buffered saline, pH 7.2, to remove most of the remaining ammonium sulphate or Carbowax and also to make the solutions suitable for further characterisation as follows:-

(v) Characterisation of fractions. The pooled fractions were characterised by immuno-electrophoresis (Williams and Grabar, 1955) and tested for antibody activity by the various serological tests already described.

(b) Immuno-electrophoresis (Williams and Grabar, 1955). This was carried out on microscope slides using the LKB apparatus* according to the instruction manual. 1:10,000 Merthiolate was added to all reagents as a bacteriostat.

(i) Buffer. The discontinuous veronal/calcium lactate buffer system, pH 8.6 of Hirschfeld (1960) was used.

(ii) Agar. 10 ml. of agar solution (1% Difco "Special Agar-Noble" in agar buffer) was poured into each

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* LKB - Produkter AB, Stockholm, Sweden.

row of three microscope slides previously fixed with an agar/glycerine mixture in plastic racks and held on a levelling table. After maturing, usually overnight, two wells and a central trough were cut on each slide using the LKB Gel punch set. The diffusion distance was 3 mm., the diameter of the wells 1 mm. and the width of the trough 2 mm.

(iii) Electrophoresis run. 5 µl. of antigen (serum or serum fraction) was applied to each well and bromophenol blue added so that the migration distance could be measured. Whole serum was run in parallel to the fractions, i.e. on the same slide, for comparison. The potential difference across the racks was 300 volts and the current flowing across each rack 6-7 milliamps. Once the albumin had migrated 18-20 mm. the run was stopped. This usually took $1\frac{1}{2}$ to $1\frac{3}{4}$ hrs.

(iv) Application of antisera. The longitudinal trough was carefully removed and 0.1 ml. of a 1 in 2 dilution in isotonic saline of the specific antiserum added - usually rabbit anti-pig-serum.

The racks of slides were then allowed to stand in a moist chamber at room temperature for 48 hrs. for diffusion and precipitation to take place before washing in two changes of isotonic saline and finally in distilled water over a period of 36-48 hrs. After drying under lint-free filter paper strip, staining was carried out to demonstrate the precipitin lines as for the immuno-diffusion technique.

10. Haematology.

Haematological examination of piglets' blood was carried out on samples which had been dispensed into dry ethylene-diamine-tetra-acetic acid (EDTA, Sequestrene) in small plastic tubes.

(a) Packed cell volume (PCV). Packed cell volume was measured using micro-haematocrit tubes, centrifuge and reader (Hawksley, London), the samples being centrifuged for 5 minutes.

(b) Haemoglobin estimation (Hb). Haemoglobin levels were estimated using the alkaline haematin method of Clegg and King (1942), using the standard solution of Gibson and Harrison (1945) for comparison on an Eel colorimeter with green filter No.624. All values were expressed in grams per 100 ml (g%).

0.05 ml of blood was washed into and well mixed with 4.95 ml N/10 NaOH. The test sample was then heated along with a sample of the standard solution in a boiling water bath for 4 min. and cooled quickly in cold water. Matching in the colorimeter was then carried out within 30 minutes, a fresh standard being used for each set of samples.

(c) Red cell counts. These were carried out on a Coulter* electronic counter (Model A, Medical) using threshold setting 10 and Current setting 5. Samples were diluted using the Coulter* Dual Diluter and the mean of three consistent counts was taken.

(d) Direct sensitisation (Coombs) Test. To detect the

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* Coulter Electronics Limited, Dunstable, Beds., England.

presence of antibody on the piglets' red cells, the direct antiglobulin (Coombs) sensitisation test (Coombs, Mourant and Race, 1946) modified so that it corresponded to the second stage of the indirect sensitisation test (4.C.ii) was carried out on these cells. This test was also recommended by Buxton, Brooksbank and Coombs (1955) for the diagnosis of haemolytic disease of newborn piglets.

Red cells from piglets were washed three times in phosphate buffered saline and a 2% suspension of cells in saline made up as already described. One drop of cells was added to one drop of rabbit anti-pig-globulin (1:50 in saline) in 50 x 7 mm tubes, mixed thoroughly and incubated at 37°C for 1 h. Results were read macroscopically as for the indirect sensitisation test. Suspensions of red cells in saline alone were included as controls for all samples.

(e) Thrombocyte counts. The platelet diluting fluid recommended by Baar (1948) was used. This was made up as follows:

Saponin*	0.25 g.
Sodium citrate	3.5 g.
Formalin (40% Formaldehyde)	1.0 ml.
Dist. aqua	ad 100.0 ml.

Baar included 0.1 g. brilliant cresyl blue as well but this was found to be unnecessary.

All dilutions were made directly into white cell diluting pipettes in the handling room of the piggery with the help of one assistant. Diluting fluid was drawn up to the 0.5 mark on the pipette and the excess wiped off. Blood,

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* BDH Chemicals Ltd., Poole, Dorset, England.

taken from piglets' ears as already described, was drawn up into the pipette until the diluting fluid reached the 1 mark, any excess again being wiped off before more diluting fluid was drawn in until the 11 mark was reached. Pipettes were then shaken a few times and laid in a rack to be taken to the laboratory for counts to be performed.

Counts were carried out in the improved Neubauer silver line platelet counting chamber*. Before filling, the pipettes were agitated for two minutes and the first half of fluid discarded. Once full, chambers were allowed to sit in moist petri dishes for 20 minutes before counting.

For counting, the red cell counting area or the rectangles at each side of it were used - i.e. either 5 groups of 16 small squares or 4 groups of 4 rectangles - in a light field microscope at 100 times magnification. The average of counts in both chambers was taken provided these did not differ from the mean by more than 10%. By multiplying by 1,000, the number of platelets per cu mm of blood was given.

A technique using the Coulter counter was also tried (Eastham, 1963) using samples taken into EDTA as anti-coagulant. However, this method was found to be more time-consuming and no good for thrombocytopenic animals, both because they had to be bled from the anterior vena cava and hence haemorrhage could not be controlled, and because in these animals the thrombocytes tended to be of widely differing sizes.

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* Hawksley, London.

11. Statistics

Results were statistically analysed by analysis of variance and "Student's" t test (Snedecor, 1965).

PRELIMINARY INVESTIGATIONS

1. Red Cell Studies

(a) Minimal disease herd. All the adult animals were blood sampled and their sera tested for red cell antibodies (Table 1). All were of group Ao except 575 which was Aa.

Table 1

Reciprocals of red cell antibody titres in the serum of adult pigs in the Minimal Disease Herd.

Pig No.	Anti-A			Iso-antibody
	D.A.	IST	Haem.	
88	4	8	4	2 (anti-Ea)
143	1	8	8	-
194	4	16	4	-
241	1	2	-	-
280	4	32	-	-
362	2	-	-	-
405	2	32	8	2 (anti-Ea)
575	-	-	-	-
630	1	4	8	-
666	1	4	8	-
668	1	16	8	-
791	1	-	2	-
977	4	16	4	-
1113	1	8	4	-
1186	1	64	8	-
Rocky	1	32	8	-
W.B.	2	8	8	-

The sera were titrated against cells from six animals by the direct agglutination, indirect sensitisation and haemolytic techniques. At first only serum dilutions 1:1 to 1:8 were tested but if a reaction was present at the latter, the serum was further diluted until the end point was reached. For example, tests on serum ex sow 194 were carried out as follows:

Direct agglutination Test.

Cell Source	Serum Dilution				Saline control (SC)
	1:1	1:2	1:4	1:8	
W.3184	V	++	+	-	-
71P16	V	++	+	-	-
T38P12	-	-	-	-	-
P20	-	-	-	-	-
RW	-	-	-	-	-
6	-	-	-	-	-

Indirect Sensitisation Test.

Cell Source	Serum Dilution						SC
	1:1	1:2	1:4	1:8	1:16	1:32	
W3184	++	++	++	+	(+)	-	-
71P16	++	++	++	+	(+)	-	-
T38P12	-	-	-	-	-	-	-
P20	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

Haemolytic Technique.

Cell Source	Serum Dilution				Controls	
	1:1	1:2	1:4	1:8	Saline	Complement
W3184	5	2	-	-	-	-
71P16	5	2	-	-	-	-
T38P12	-	-	-	-	-	-
P20	-	-	-	-	-	-
RW	-	-	-	-	-	-
6	-	-	-	-	-	-

From this it was fairly obvious that anti-A alone was present in this serum because the only two animals whose red cells reacted with the serum were of group Aa. However,

checkerboard analysis was carried out as follows for confirmation.

Direct Agglutination Test.

Cell Type	Cell Source	Serum ex 194					
		Unabsorbed	Absorbed				SC
			W3184	71P16	T38P12	P20	
Aa	W3184	V	-	-	V	V	-
Aa	71P16	V	-	-	V	V	-
Ao	T38P12	-	-	-	-	-	-
Ao	P20	-	-	-	-	-	-

Indirect Sensitisation Test.

Cell Type	Cell Source	Serum ex 194					
		Unabsorbed	Absorbed				SC
			W3184	71P16	T38P12	P20	
Aa	W3184	++	-	-	++	++	-
Aa	71P16	++	-	-	++	++	-
Ao	T38P12	-	-	-	-	-	-
Ao	P20	-	-	-	-	-	-

Similar results were obtained with all the others except sows 88 and 405.

Sow 88

Direct Agglutination

Cell Type	Cell Source	Serum Dilution					SC
		1:1	1:2	1:4	1:8	1:16	
Aa	W3184	V	++	+	(+)	-	-
Aa	71P16	V	++	+	-	-	-
Ao	T38P12	++	+	-	-	-	-
Ao	P20	-	-	-	-	-	-
Ao	RW	-	-	-	-	-	-
Ao	6	-	-	-	-	-	-

Indirect Sensitisation Test.

Cell Type	Cell Source	Serum Dilution 1:1	1:2	1:4	1:8	1:16	1:32	SC
Aa	W3184	++	++	++	+	+	-	-
Aa	71P16	++	++	+(+)	+	-	-	-
Ao	T38P12	++	+	-	-	-	-	-
Ao	P20	-	-	-	-	-	-	-
Ao	RW	-	-	-	-	-	-	-
Ao	6	-	-	-	-	-	-	-

Haemolytic Technique.

Cell Type	Cell Source	Serum Dilution 1:1	1:2	1:4	1:8	Controls Saline	Complement
Aa	W3184	5	5	3	-	-	-
Aa	71P16	5	5	3	-	-	-
Ao	T38P12	-	-	-	-	-	-
Ao	P20	-	-	-	-	-	-
Ao	RW	-	-	-	-	-	-
Ao	6	-	-	-	-	-	-

Thus a reaction was present between this serum and Ao cells (T38P12) and for the first time evidence of an antibody other than anti-A was present. This antibody was confirmed as being anti-Ea by checkerboard analysis as follows:-

Direct Agglutination Test.

Cell Type	Cell Source	Serum ex 88 Unabsorbed	Absorbed W3184	71P16	T38P12	P20	SC
Aa Ea	W3184	V	-	++	++	++	-
Aa -	71P16	++	-	-	++	++	-
Ao Ea	T38P12	++	-	++	-	++	-
Ao -	P20	-	-	-	-	-	-

Indirect Sensitisation Test.

Cell Type	Cell Source	Serum ex 88					SC
		Unabsorbed	Absorbed				
			W3184	71P16	T38P12	P20	
Aa Ea	W3184	++	-	++	++	++	-
Aa -	71P16	++	-	-	++	++	-
Ao Ea	T38P12	++	-	++	-	++	-
Ao -	P20	-	-	-	-	-	-

Similarly anti-Ea was found to be present in the serum of 405 at a titre of 1:2. The full breeding histories of 88 and 405 were as shown in Table 2.

Of these sires, 182 was still available for blood typing, as were the four offspring listed. These five animals were typed for factor Ea using reagent 71 of the Blood Group Research Unit by the routine direct agglutinating method (Table 3).

Table 2 Breeding histories of sows 88 and 405.

	Litter No.	Sire Ear No.	Offspring Ear No.
Sow 88	1	82	280
	2	110	
	3	110	
	4	19	
	5	182	
	6	187	
	7	182	
	8	182	
Sow 405	1	139	791
	2	187	977
	3	187	1113
	4	182	
	5	182	

Table 3 Typing of pigs for blood group factor Ea

Pig No.	Ea (Reagent 71)
182	-
280	-
791	-
977	+
1113	-

Hence 977 was the only Ea positive animal available and, as her dam, 405, and her full sister, 1113, were Ea negative, her sire, No. 187 must have been heterozygous for this factor. As both 88 and 405 had been mated to this boar in the past, there was evidence that both had given birth to Ea positive piglets. Both were at this time in-pig to boar No. 182, i.e. an Ea negative animal, and it was decided to follow the antibody titres throughout pregnancy and after farrowing to observe any fluctuations.

For the subsequent pregnancy, both sows were artificially inseminated with semen from a boar, Balhary Field Marshall 2, which was standing at the Selby A.I. centre, and which was Ea positive. Similarly two Ea negative gilts, Nos. 1347 and 1350, were artificially inseminated with semen from the same boar to see if anti-Ea would be produced in their serum after carrying Ea positive piglets. Full blood types of these animals were known (Materials and Methods, Table I). The genotypes of these animals in the E system where the boar was carrying factor(s) which the sows and gilts did not have were therefore known (Table 4).

Table 4

Pig No.	Sex	E System Genotype
88	F	bdg/bdg
405	F	bdg/edg
1113	F	bdg/edg
1347	F	bdg/edg
1350	F	bdg/edg
BFM 2	M	bdg/aeg

As Balhary Field Marshall 2 was heterozygous for the Ea factor, both Ea positive and Ea negative piglets could be expected in the litters of 88 and 405. Therefore the Ea negative piglets could act as controls for the study of the effects of anti-Ea on the red cells of their Ea positive litter mates as discussed under Haematology.

(b) Blood group research unit herd. Sows 301 and 302 which were found to have red cell iso-antibodies present in their sera were brought into the blood group research unit piggery for further studies.

Sow 301 This animal was nursing her second litter (11 piglets) when purchased.

Antibody levels in her serum were as follows:-

Direct Agglutination Test.

Cell Source	Serum Dilution				SC
	1:1	1:2	1:4	1:8	
W3184	V	V	+(+)	-	-
PY4731	V	++	+	-	-
T38P12	V	+(+)	-	-	-
P20	V	+	-	-	-
RW	V	+	-	-	-
6	V	+	-	-	-
L413	-	-	-	-	-

Indirect Sensitisation Test.

Cell Source	Serum Dilution						SC
	1:1	1:2	1:4	1:8	1:512	1:1024	
W3184	++	++	++	++	++	-	-
PY4731	++	++	++	++	++	-	-
T38P12	-	-	-	-	-	-	-
P20	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
L413	-	-	-	-	-	-	-

Haemolytic Technique.

Cell Source	Serum Dilution					Controls	
	1:1	1:2	1:4	1:8	1:16	SC	CC
W3184	1	4	2	2	-	-	-
PY4731	1	4	2	2	-	-	-
T38P12	-	-	-	-	-	-	-
P20	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
L413	-	-	-	-	-	-	-

The indirect sensitisation and haemolytic reactions were taken as anti-A and the direct agglutinating iso-antibodies present were characterised by checkerboard analysis as follows:

Cell Source	Relevant Cell Type	Serum 301								SC
		Unabsorbed	Absorbed Red Cells ex							
			W3184	PY4731	T38P12	P20	RW	6	L413	
W3184	Aa,Ea,Kb	V	-	+	++	++	++	++	V	-
PY4731	Aa - Kb	V	-	-	++	++	++	++	V	-
T38P12	Ao,Ea,Kb	V	-	+	-	+	+	+	V	-
P20	Ao - Kb	V	-	-	-	-	-	-	V	-
RW	Ao - Kb	V	-	-	-	-	-	-	V	-
6	Ao - Kb	V	-	-	-	-	-	-	V	-
L413	Ao - -	-	-	-	-	-	-	-	-	-

From this it was concluded that anti-Ea was present as well as anti-A. However, the reactions between the serum and P20, RW and 6 still remained obscure. Anti-Kb was a possibility, the Kb antigen being common to the red cells all through but not being carried by those of L413. Therefore a secondary checkerboard was set up including an animal which was Aa positive, Ea negative, and Kb negative (No.72).

Cell Source	Relevant Cell Type	Serum 301							SC
		Unabsorbed	Absorbed Red Cells ex						
			W3184	72	T38P12	P20	RW	6	L413	
W3184	Aa Ea Kb	V	-	++	++	++	++	++	V	-
72	Aa - -	V	-	-	+	++	++	++	V	-
T38P12	Ao Ea Kb	V	-	++	-	+	+	+	V	-
P20	Ao - Kb	V	-	++	-	-	-	-	V	-
RW	Ao - Kb	V	-	++	-	-	-	-	V	-
6	Ao - Kb	V	-	++	-	-	-	-	V	-
L413	Ao - -	-	-	-	-	-	-	-	-	-

Therefore it was concluded that the iso-antibodies, anti-Ea and anti-Kb, were present in this serum as well as the natural antibody, anti-A. There was no history of injections of pig red cells or other blood products but the present litter of 11 piglets was available for red cell typing (Table 5). From this it was ascertained that both Ea positive and Kb positive piglets had been born and the possibility existed of iso-immunisation having taken place by these piglets' red cells during pregnancy or at parturition.

This sow was again mated to a boar which was Ea positive, Kb positive (Balhary F.M.2). As full blood types were known (Materials and Methods, Table I), the genotypes for the incompatible systems could be worked out (Table 6).

Table 5. Red cell typing of litter ex 301.

Pig No.	A		D		E						F		G		I		K			L		Gb/Sc
	a	o	a	b	a	b	d	e	f	g	a	b	a	b	a	b	a	b	d	a	g	16
1	-	-	+	/	+	+	+	+	-	+	-	/	-	+	/	+	+	+	+	-	+	-
2	-	+	+	/	-	+	+	+	-	+	-	/	+	+	/	-	+	+	+	-	+	-
3	-	+	+	/	+	+	+	+	-	+	-	/	+	+	/	+	+	+	+	-	+	-
4	-	+	+	/	-	+	+	+	-	+	-	/	-	+	/	+	+	-	+	-	+	-
5	-	-	+	/	-	+	+	+	-	+	-	/	-	+	/	-	+	+	+	-	+	-
6	-	-	+	/	+	+	+	+	-	+	-	/	-	+	/	+	+	+	+	-	+	-
7	-	+	+	/	-	+	+	+	-	+	-	/	-	+	/	+	-	+	+	-	+	-
8	-	-	+	/	-	+	+	+	-	+	-	/	-	+	/	+	-	+	+	-	+	-
9	-	-	+	/	+	+	+	+	-	+	-	/	-	+	/	-	+	+	+	-	+	-
10	-	+	+	/	-	+	+	+	-	+	-	/	-	+	/	+	+	+	+	-	+	-
11	-	+	+	/	-	+	+	+	-	+	-	/	+	+	/	+	+	-	+	-	+	-
Sow 301	-	+	-	/	-	+	+	-	-	+	-	/	-	+	/	+	+	-	+	-	+	-
Boar	-	+	+	/	+	-	+	+	-	+	-	/	+	+	/	-	-	+	+	-	+	-

Table 6

Pig No.	Sex	E System	K System
301	F	bdg/bdg	ade/-
BFM2	M	aeg/bdg	-/b

Subsequently she was mated to a boar, No. 8, which was Ea positive, Kb positive. The full red cell types were known (Materials and Methods, Table I) and the genotypes in the incompatible systems determined (Table 7).

Table 7

Pig No.	Sex	E System	K System
301	F	bdg/bdg	ade/-
8	M	aeg/edg	ace/b

Finally sow 301 was mated to a boar, No.312, which was Ea negative, Kb negative and the levels of anti-Ea and anti-Kb followed throughout pregnancy and after parturition.

Sow 302. This animal was in the late stages of her second pregnancy when first sampled. Antibody levels at this time were as follows:-

Direct Agglutination

Cell Source	Serum Dilutions						SC
	1:1	1:2	1:4	1:8	1:16	1:32	
W3184	V	V	++	+(+)	+	-	-
PY4731	V	V	+(+)	+	-	-	-
T38P12	++	W	-	-	-	-	-
P20	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

Indirect Sensitisation Test.

Cell Source	Serum Dilutions									SC
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
W3184	++	++	++	++	++	++	++	+	-	-
PY4731	++	++	++	++	++	++	++	+	-	-
T38P12	-	-	-	-	-	-	-	-	-	-
P20	-	-	-	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-

Haemolytic Technique.

Cell Source	Serum Dilutions					Controls	
	1:1	1:2	1:4	1:8	1:16	SC	CC
W3184	2	2	3	3	-	-	-
PY4731	2	2	3	3	-	-	-
T38P12	-	-	-	-	-	-	-
P20	-	-	-	-	-	-	-
RW	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

The direct agglutinating iso-antibody was proved to be anti-Ea by checkerboard analysis as follows:-

Cell Source	Relevant Cell Type	Serum 302							
		Unabsorbed	Absorbed Red Cells ex						SC
			W3184	PY4731	T38P12	P20	RW	6	
W3184	Aa Ea	V	-	++	V	V	V	V	-
PY4731	Aa -	V	-	-	V	V	V	V	-
T38P12	Ao Ea	++	-	++	-	++	++	++	-
P20	Ao -	-	-	-	-	-	-	-	-
RW	Ao -	-	-	-	-	-	-	-	-
6	Ao -	-	-	-	-	-	-	-	-

Hence anti-A and anti-Ea were present in this serum. The sow was known to be in-pig to an Ea positive boar for the second time and red cell antibody titres were followed up to and after parturition.

After the piglets were weaned 302 was artificially inseminated with semen from Balhary FM 2 - full red cell typing of both animals had been carried out (Materials and Methods, Table I) and the genotypes of incompatible systems determined (Table 8).

Table 8

Pig No.	Sex	E System	K System
302	F	bdg/edg	ade/-
BFM 2	M	bdg/aeg	-/b

For a subsequent litter 302 was served by 311 - full red cell typing of both animals had been carried out (Materials and Methods, Table I) and the genotypes of incompatible systems determined (Table 9).

Table 9

Pig No.	Sex	K System	L System	E System
302	F	ade/-	bcg/bcg	bdgk/edghk
311	M	-/b	bcgi/adhjl	edgh/edfh

2. Thrombocyte Studies

(a) Dams of litters affected with thrombocytopenic purpura. Four sows which had produced litters affected with thrombocytopenic purpura were purchased along with the boars to which two of them had been mated. Histories of these animals were:-

Sow 30: This was a Welsh sow which had had five litters as follows:-

Farrowing	Boar		Number of Piglets	
	Identity	Breed	Born	Reared
1	Victor	Welsh	12	9
2	David	Large White	12	11
3	David	Large White	14	14
4	David	Large White	14	14
5	N.O.B.	Large White	15	4

The poor survival rate in the fifth litter was accounted for entirely by losses due to thrombocytopenic purpura, 10 piglets dying at 3 days of age and one at 3 weeks. When purchased, this sow was again in-pig to boar N.O.B. and thrombocyte counts were carried out on members of the resulting litter as earlier described.

After weaning, she was artificially inseminated with semen from a Large White boar chosen at random and the thrombocyte counts of members of the litter produced followed as before.

Sow 304: This Landrace sow was purchased along with the Landrace boar (No. 303) to which she had been mated on five consecutive occasions. Her breeding history was as follows:-

Farrowing	Number of Piglets	
	Born	Reared
1	7	7
2	9	7
3	8	5
4	8	1

Litters 3 and 4 had been affected clinically with thrombocytopenic purpura. Thrombocyte counts of members of litter No. 5 were monitored as before.

Unfortunately the boar, 303, had to be put down because of a hip lameness and this precluded a further mating.

Sow 305: This cross Landrace sow was purchased along with the five surviving piglets of a litter which had been affected with thrombocytopenic purpura and the sire of

the litter, a Large White boar, No. 8. Four litters had been produced, all to boar No. 8. The full breeding history was as follows:-

Farrowing	Number of Piglets	
	Born	Reared
1	6	6
2	12	3 (9 stillborn)
3	12	10
4	12	5

After weaning, 305 was mated to boar No. 8 again.

Of the fifth litter from 305, half were left with her and half were fostered on to another sow, No.1083, which had farrowed 12 h. earlier and which had successfully reared one previous litter. Similarly half of 1083's litter were fostered on to 305 and half left with their dam. Thrombocyte counts of all these piglets were followed as before.

Subsequently 305 was mated to her son, 312, an affected member of her fourth litter, and thrombocyte counts were carried out on members of the resulting litter.

Sow 343, was of the Landrace breed and had had two normal litters before the one in which three piglets had died of thrombocytopenic purpura. She was mated to the same Landrace boar for a fourth time and the thrombocyte counts of the piglets produced monitored as before.

(b) Survivors of thrombocytopenic litters. Two unaffected female survivors, 306 and 307, and one affected female survivor, 309, of a litter which had suffered from

thrombocytopenic purpura were mated to the affected male survivor, 311 of the same litter. In view of the iso-immunisation theory for the pathogenosis of thrombocytopenic purpura put forward by other workers (Stormorken et al., 1963; Saunders and Kinch, 1968), it was presumed that, in this case, the gilts 306 and 307, were of the same platelet type as the dam and 309 and 311 were of similar type to the sire i.e. heterozygous for the factor to which antibodies had been produced.

309 was retained as a control for 306 and 307 which were injected with platelets from 311 as follows:-

Platelets were isolated in as pure a suspension as possible in the usual manner - less than 5 red and white cells per mm^3. An emulsion was made with 5 ml of platelet suspension (5×10^5 platelets per mm^3) and 5 ml of Freund's complete adjuvant and 306 and 307 were each given intramuscular injections of this quantity at the time of service. This procedure was repeated after fourteen days.

306 was given a further injection of 5 ml platelet suspension alone intravenously one month later and 307 given a similar injection two weeks before farrowing.

For their second litters, 306 was given a similar intravenous injection of platelet suspension alone seven days prepartum while 307 was given no further injections.

For their third litters, 306 was given intravenous injections of platelet suspension midway through pregnancy and at parturition; 307 was given similar injections at

service and at parturition.

311 was the sire of all litters and the donor of platelets for all the immunisations.

Thrombocyte counts were followed in all members of all litters as already described.

EXPERIMENTAL RESULTS

1. The development of iso-antibodies to red cell antigens in sows' sera without artificial stimulation

The following experiments were designed to elucidate the occurrence of antibodies to red cell antigens other than anti-A in the sera of sows and to investigate the hypothesis that iso-immunisation might occur by foetal red cells either during pregnancy or at parturition.

The animals studied were those in the Minimal Disease herd, 88, 405, 1113, 1347 and 1350, and those in the Blood Group Research Unit herd, 301 and 302. Full life histories of all these animals were available and there was no evidence that any of them had been injected with any porcine tissues or body fluids which might have been contaminated with red cell antigens or which might otherwise have led to the production of antibodies to these antigens.

Sow 88. When first studied, this sow was in-pig to an Ea negative animal. The anti-Ea titres, as detected by the reactions between her serum and red cells from T38P12, were followed throughout the last month of pregnancy and after parturition.

No marked changes in these levels were observed over this period, only slight fluctuations (Fig. 1.1; Appendix I, Table 1.a). Red cell typing of the litter confirmed that all the piglets were Ea negative.

After weaning, the sow was artificially inseminated with semen from an Ea positive boar, Balhary Field Marshall 2 (BFM2). The red cell antibody levels were monitored throughout pregnancy and after parturition using red cells

from four animals chosen so that anti-A plus anti-Ea, anti-A alone, and anti-Ea alone, could each be estimated and the possible occurrence of any other iso-antibodies detected. In addition the antibody titres to red cells from BFM2 were followed for a part of the time using red cells from that animal.

No antibodies were detected to the red cells of the Ao, Ea negative animal while titres to the Aa, Ea negative animal (anti-A) remained constant (Appendix I, Table 1a). The titres to red cells from BFM2 remained constant throughout pregnancy right up to parturition after which there was a sharp rise reaching a peak at seventeen days post partum (Fig. 1.2; Appendix I, Table 1.a). These levels were maintained for about two weeks after which there was a gradual decline. The titres to red cells from the other Ao, Ea positive animal followed a similar pattern (Fig. 1.1; Appendix I, Table 1.a) and, after this, it was considered satisfactory to monitor the anti-Ea levels with red cells from this animal as BFM2 was not readily accessible. Titres gradually fell up to seventeen weeks post partum when observations ceased.

The combined titres of anti-A and anti-Ea followed a similar pattern to anti-Ea alone but were at a higher level and the increase after parturition was not so clearly marked, the titre level of anti-Ea being apparently masked by the anti-A reaction (Appendix I, Table 1.a).

A checkerboard analysis of the antibodies present at the height of the reaction confirmed that the increase had in fact

been in anti-Ea as far as could be determined from the panel of animals used (Appendix I, Table 1.b).

The 12 piglets born were red cell typed by the reagents used routinely by the Blood Group Research Unit (Appendix I, Table 1.c); nine were Ea positive, three Ea negative.

Sow 405. Studies on this animal were carried out in a similar fashion. (Fig. 1.3; Appendix I, Table 2.a).

When mated to an Ea negative boar, the anti-Ea levels did not vary much either during pregnancy or after parturition when only Ea negative piglets were born.

After weaning, 405 was artificially inseminated with semen from BFM2. The levels of anti-Ea, as monitored by the reactions between her serum and cells from T38P12, remained constant throughout pregnancy right up to parturition, after which there was a distinct rise in titre both by the direct agglutination and indirect sensitisation techniques, reaching a peak at ten days post partum followed by a gradual decline. The combined, anti-A and anti-Ea levels showed a similar pattern but were more difficult to interpret due to marked fluctuations in anti-A. Throughout the whole period of study, the latter, while frequently varying in titre, showed no definite trend and no reactions were demonstrated between the serum of 405 and the red cells from the Ao, Ea negative animal.

Antibody analysis, when the titres were at their peak, confirmed that the increase after the second parturition was due to anti-Ea (Appendix I, Table 2.b) and red cell typing

of the litter born at this time demonstrated ten Ea positive piglets and five Ea negative ones (Appendix I, Table 2.c).

Sow 301. This animal had low levels of anti-Ea and anti-Kb as well as anti-A in her serum and was artificially inseminated with semen from BFM2 which was both Ea and Kb positive. The serum anti red cell antibody levels were monitored with a panel of five animals (Appendix I, Table 3.a) throughout pregnancy and for twelve weeks after parturition.

Anti-A remained stable throughout the whole period of study and no antibodies were detected to red cells of the animal which was Ao, Ea negative, Kb negative. The combined levels of anti-Ea and anti-Kb, estimated with cells from T38P12, remained stable for the first eight weeks of pregnancy, after which time there was a sudden increase in both the direct agglutinating and the indirect sensitising titres reaching a peak two weeks later (Fig. 1.4). These high levels were maintained for about five weeks before a distinct drop occurred at parturition. Although the initial levels were never reached, there was a continual drop up to twelve weeks post partum, apart from a slight rise immediately after parturition.

The anti-Kb levels on their own determined with cells from RW, showed a much less pronounced rise (Fig. 1.4), the direct agglutinating titre showing a slight increase at the same time as the combined anti-Ea and anti-Kb one and falling to a relatively low level by twelve weeks post partum.

The indirect sensitising reaction was completely absent in the initial stages but became apparent about three weeks pre-partum reaching a peak two weeks later only to drop at parturition and recover peak levels after a further one and a half to two weeks. Finally, it also gradually dropped up to twelve weeks post partum.

Unfortunately, as no animal which was Ao, Ea positive, Kb negative, was available, it was not possible to follow the anti-Ea levels on their own.

Antibodies present at the peak levels were again characterised by absorption and checkerboard analysis of the results and those present were confirmed as being anti-Ea and anti-Kb along with anti-A (Appendix I, Table 3.b).

Typing of the litter confirmed that both Ea positive and Kb positive piglets had been born (Appendix I, Table 3.c).

Hence it was seen that a definite increase in red cell iso-antibodies occurred at about eight weeks pre-partum in 301 during a pregnancy in which piglets carrying the corresponding red cell antigens were borne. Most of the increase occurred in the anti-Ea level while the anti-Kb one showed only a slight increase, the direct agglutinating antibodies becoming apparent before the indirect sensitising ones.

301 was again mated to an Ea-positive, Kb positive boar, 8, and the observations repeated (Appendix I, Table 4.a).

Similar responses in antibody levels were noted to the previous mating. The combined anti-Ea and anti-Kb levels, as determined by the reactions with red cells from 8, rose

about seven to eight weeks before farrowing (Fig. 1.5) and reached a peak three to four weeks later only to drop at parturition and up to three weeks post partum when observations ceased. This time, however, the anti-Kb titres as determined by reactions with red cells both from RW and 191 reached the same levels as the combined anti-Ea, and anti-Kb and ran in parallel with the latter (Fig. 1.5). Hence, on this occasion, the anti-Kb response was apparently greater than the anti-Ea one. The direct agglutinating and indirect sensitising reactions fell off simultaneously.

The iso-antibodies present at the peak of the response were confirmed as being anti-Ea and anti-Kb using cells from a limited panel of animals (Appendix I, Table 4.b) and of nine piglets born, three were Ea positive and four Kb positive (Appendix I, Table 4.c.).

301 was mated for a third time to 312 which was Ea negative, Kb negative and the levels of anti-Ea and anti-Kb followed throughout pregnancy up to four to five weeks post partum (Appendix I, Table 5.a).

The direct agglutinating titres of both anti-Ea combined with anti-Kb, and anti-Kb alone gradually decreased over the whole period of study (Fig. 1.6). The indirect sensitising titres, on the other hand, fluctuated more although the overall trend was again for a decrease in titres. A sharp drop was apparent at parturition but the levels soon recovered to just below the preparturition ones (Fig. 1.6). There was, therefore, in this case, no apparent increase in titres of anti-Ea or anti-Kb.

Red cell typing of the litter confirmed that all five piglets born were negative for factors Ea and Kb (Appendix I, Table 5.b).

Sow 302. When purchased, 302 was already in-pig to an Ea-positive boar. The antibody levels were screened during the last two weeks of pregnancy and for nine weeks post partum with red cells from five animals (Appendix I, Table 6.a).

After parturition there was a dramatic increase in titre of anti-Ea, both by the direct agglutination and indirect sensitisation techniques (Fig. 1.7). Anti-Kb also appeared as indicated by the reactions with red cells from RW. Both levels had dropped by seven weeks post partum.

The antibodies present were confirmed as anti-Ea and anti-Kb by absorption and checkerboard analysis using red cells from a panel of six animals (Appendix I, Table 6.b), while red cell typing confirmed the presence of both Ea positive and Kb positive piglets (Appendix I, Table 6.c).

302 was then inseminated with semen from BFM2 and the serum antibody levels followed throughout pregnancy and after parturition (Appendix I, Table 7.a). Levels of both the combined anti-Ea and anti-Kb, as indicated by reactions with cells from T38P12, and the anti-Kb above, as indicated by reactions with cells from RW, remained constant throughout pregnancy (Fig. 1.7). After parturition there was a marked rise in both titres to levels much greater than at the previous parturition, reaching their peaks at one to

two weeks post partum and falling off thereafter gradually down to ten weeks post partum when observations ceased. No reactions were present to the cells of L413 which was Ao, Ea negative, Kb negative, and, as far as could be determined from the panel of animals used, no further antibodies were produced.

The antibodies present at the peak period were confirmed as anti-Ea and anti-Kb (Appendix I, Table 7.b) and red cell typing of the litter demonstrated the presence of eight Ea positive piglets and ten Kb positive ones (Appendix I, Table 7.c).

302 was then mated to 311 which was Aa, Ea negative, Kb positive, to see whether anti-Kb would be stimulated at the subsequent parturition without the anti-Ea being affected.

The levels of the combined anti-Ea and anti-Kb and the anti-Kb alone remained more or less constant throughout pregnancy (Fig. 1.8 a and b). After parturition there was a sharp rise in the anti-Kb indirect sensitising antibody titre though the direct agglutinating one was little affected. No such response was observed with the anti-Ea combined with the anti-Kb, and the former had apparently been unaffected but had masked the anti-Kb response. When tested with cells from the sire of the litter, 311, a sharp rise was observed after parturition (Fig. 1.8 a and b) reaching a peak at a half to one and a half weeks post partum before quickly falling off. However, a reaction was also apparent against cells from L412 over the same

period, especially by the indirect sensitisation test.

Analysis of the antibodies present at the peak level using red cells from a panel of six animals (Appendix I, Table 8.b) indicated that the iso-antibodies present were anti-Ea and anti-Kb as expected, along with a new one, anti-Ef. As the Ef antigen was carried by the red cells of 311, the reaction with cells from it included anti-Kb and anti-Ef as well as anti-A. There was no apparent rise in anti-Ea in this case.

Red cell typing of the piglets confirmed the presence of ones which were Ef positive, Kb positive and none which were Ea positive (Appendix I, Table 8.c).

Therefore in this case where Ea negative, Ef positive, Kb positive piglets were born, the production of anti-Ef and anti-Kb was stimulated in the dam while there was no apparent boost in the anti-Ea titre.

Gilts 1347 and 1350 and Sow 1113. Two maiden gilts, 1347 and 1350, and one sow, 1113, which were all Ea negative, and in whose sera there were no iso-antibodies detectable, were artificially inseminated with semen from BFM2.

No iso-antibodies were detectable in their sera throughout pregnancy. One week after parturition, anti-Ea was detected in the sera of both 1350 and 1113. In the latter the highest levels were reached after another week and maintained for two weeks before falling off, while in the former the same level was detected until observations ceased at four weeks post partum (Appendix I, Table 9.a). In both

cases anti-Ea was detected only by the direct agglutinating techniques and confirmed by checkerboard analysis of antibody absorption results (Appendix I, Table 9.b).

In the serum of 1347 no iso-antibodies were detectable apart from a slight direct agglutinating reaction at one and two weeks post partum (Appendix I, Table 9.b). However, this was too weak to be confirmed by usual techniques for antibody analysis.

Hence, in the sera of two out of these three animals, anti-Ea was produced after they had given birth to Ea positive piglets.

Conclusions

These findings support the hypothesis that iso-immunisation of sows by foetal red cells can occur, and, in the animals studied, the possibility of vaccination with porcine erythrocytes in any form could be excluded.

Anti-Ea was the antibody most studied and in three sows, it reached its highest level around ten to fourteen days after farrowing. In one of the gilts and in another sow, in both of which no iso-antibodies had previously been detected, anti-Ea was also present for the first time after giving birth to Ea positive piglets.

These observations are consistent with immunisation of the dam having taken place at or around parturition, possibly by leakage of foetal red cell antigens into the maternal circulation. That this rise is not simply physiological is shown by the fact that, when mated to Ea

negative boars, no rise in titre occurred in the two sows in the Minimal Disease herd. Similarly, in the case of 302, when mated to a boar which was Ea negative and Kb positive, no rise was noted in the anti-Ea titre after parturition but anti-Kb was boosted and a new antibody, anti-Ef, appeared, the corresponding antigen also being present on the boar's red cells.

In one animal, 301, the rise in anti-red cell titre occurred one month before parturition, possibly due to some breakdown in the placental barrier at this time with consequent spillage of foetal red cell antigens into the maternal circulation. Further, in this animal, anti-Ea and anti-Kb were the antibodies boosted and the effect was repeated during the subsequent pregnancy to another Ea positive, Kb positive boar. However, when mated to a boar which was negative for both these factors, no boost of the titres occurred either during pregnancy or after parturition, thus adding further evidence to the hypothesis that iso-immunisation by the foetal red cell antigens was occurring in the cases where an increase in serum antibody level were noted.

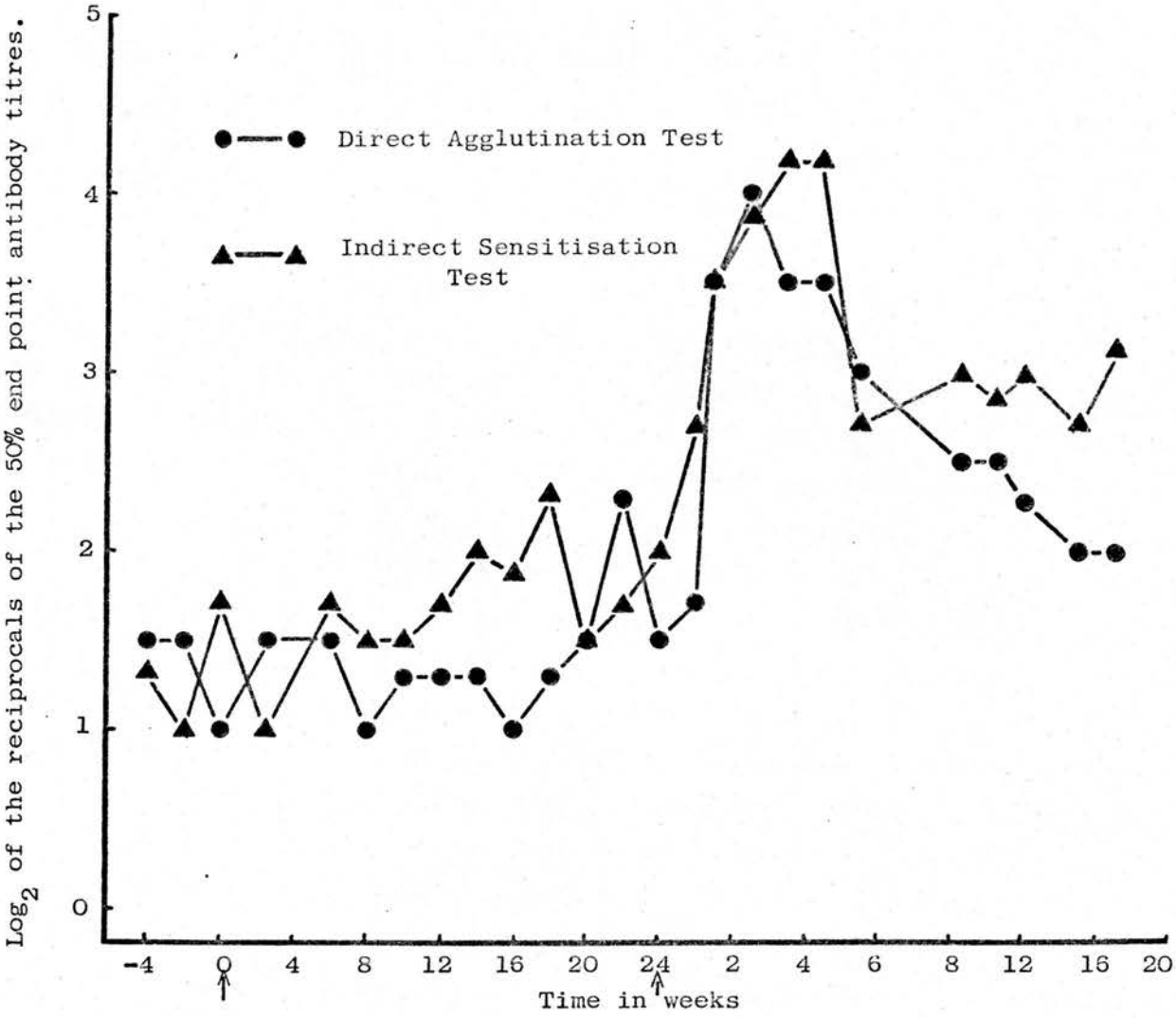


Fig. 1.1 Titres of anti-Ea in serial serum samples from 88 during pregnancy and after parturition when mated to (1) an Ea negative boar (Parturition at week 0) followed by (2) an Ea positive boar, BFM2 (Parturition at Week 24) as monitored with red cells from T38P12.

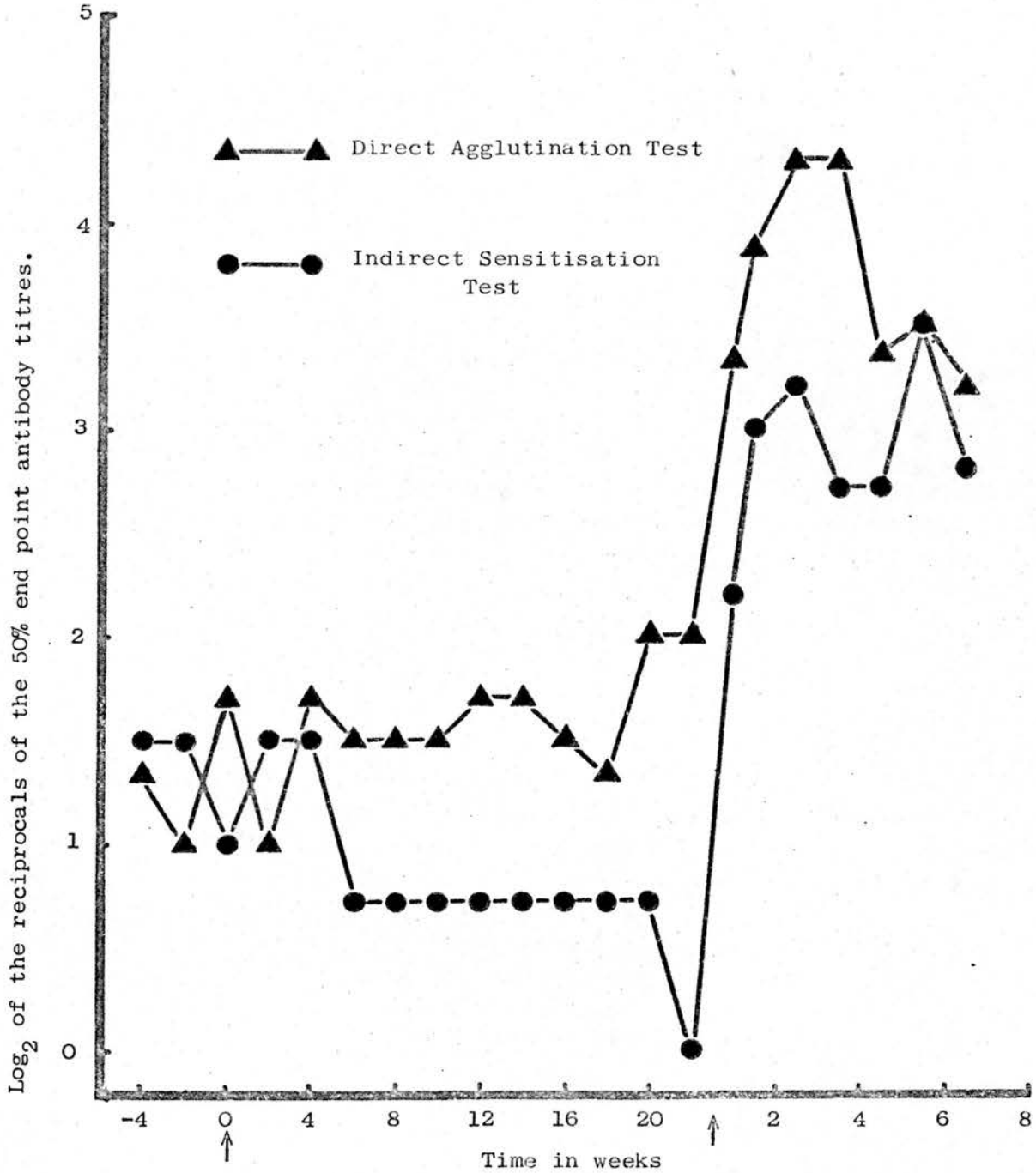


Fig.1.2 Titres of antibodies to red cells from BFM2 (anti-Ea) in serial serum samples from sow 88 during pregnancy and after parturition when mated to (1) an Ea negative boar (parturition at week 0) followed by (2) BFM2 (parturition at week 24).

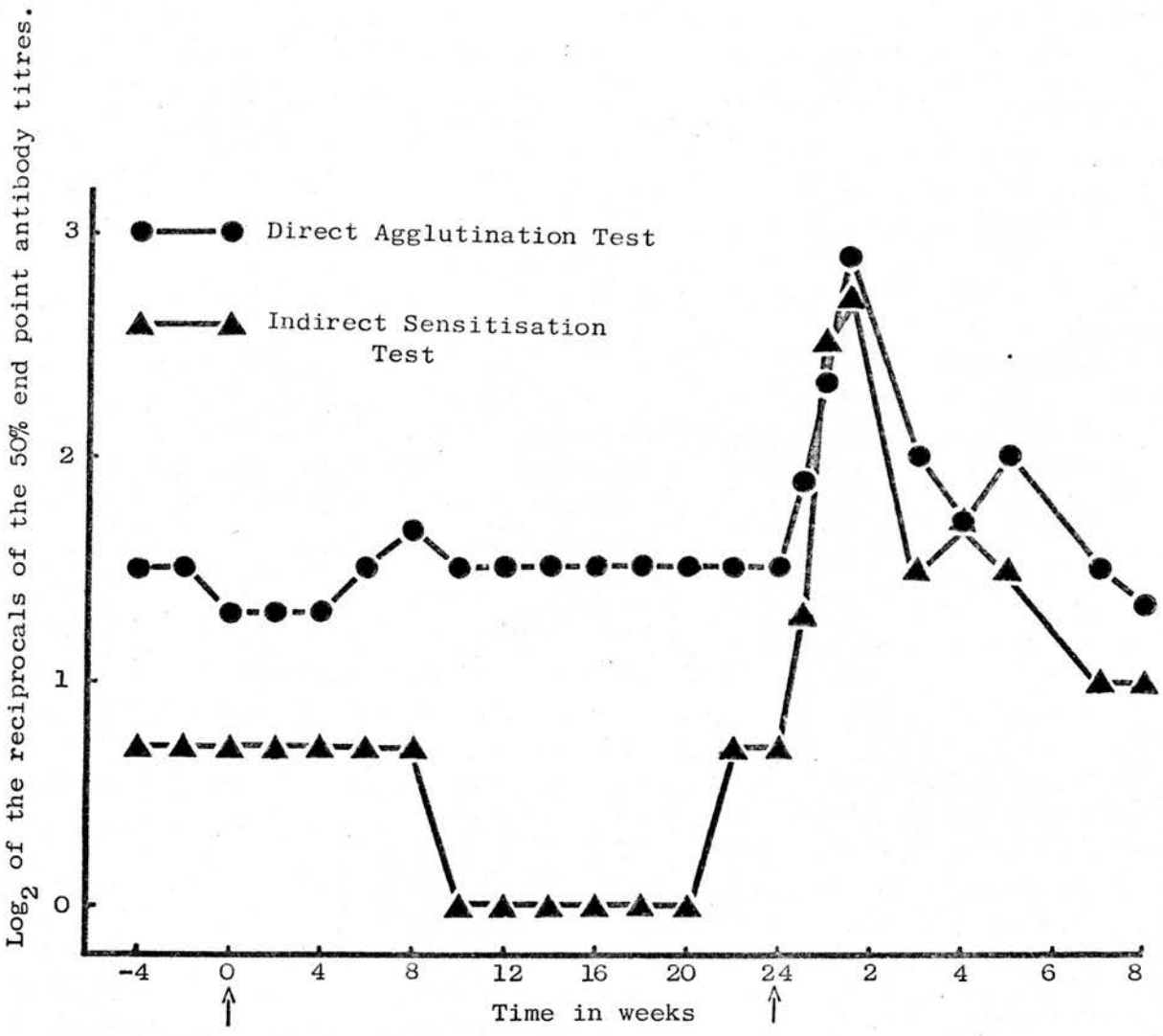


Fig.1.3 Titres of anti-Ea in serial serum samples from sow 405 during pregnancy and after parturition when mated to (1) an Ea negative boar (parturition at week 0) followed by (2) an Ea positive boar, BFM2 (parturition at week 24).

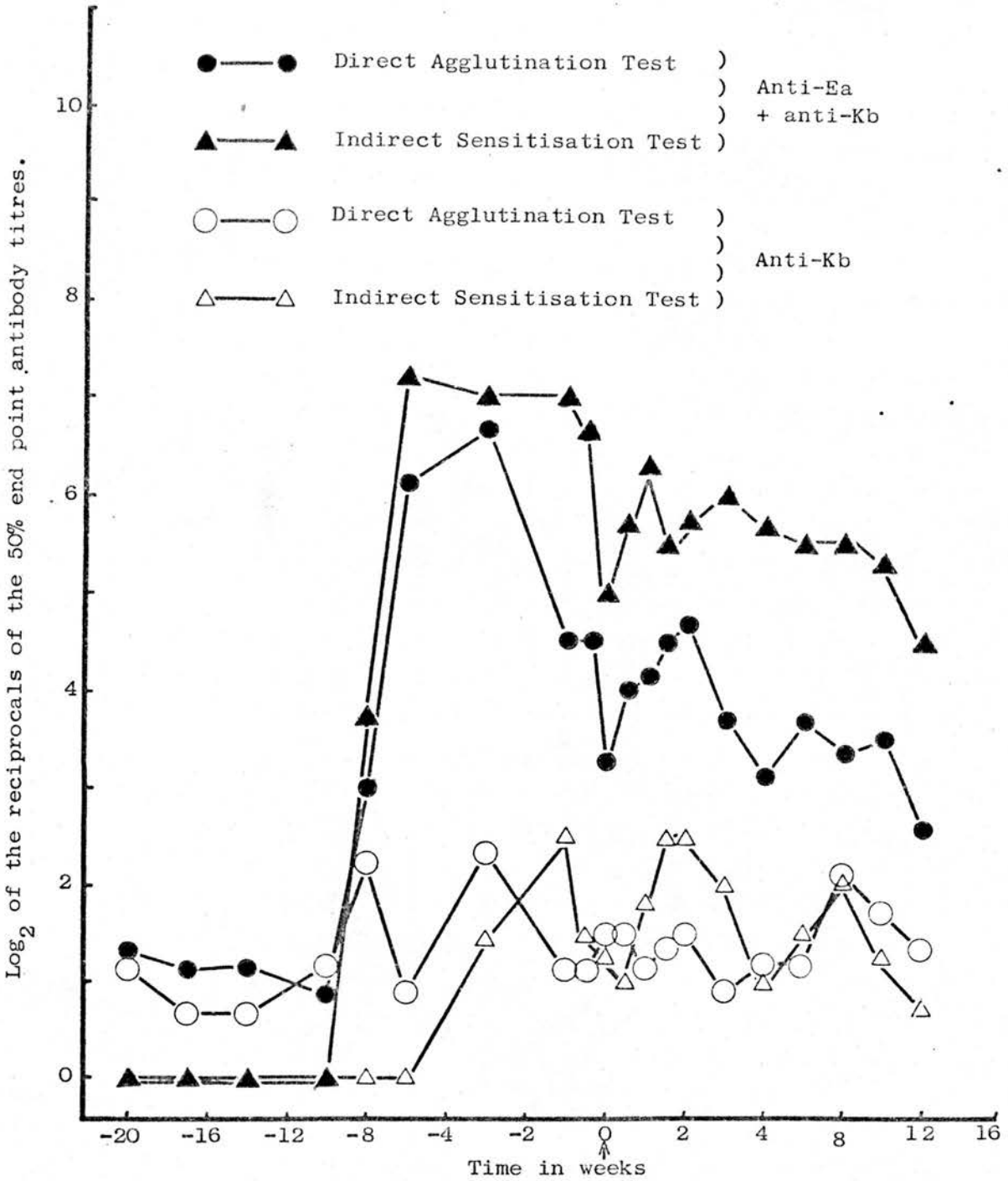


Fig.1.4 Antibody titres in serial serum samples from sow 301 during pregnancy and after parturition to BFM2 (parturition at week 0), as monitored by red cells from T38P12 (anti-Ea + anti-Kb) and RW (anti-Kb).

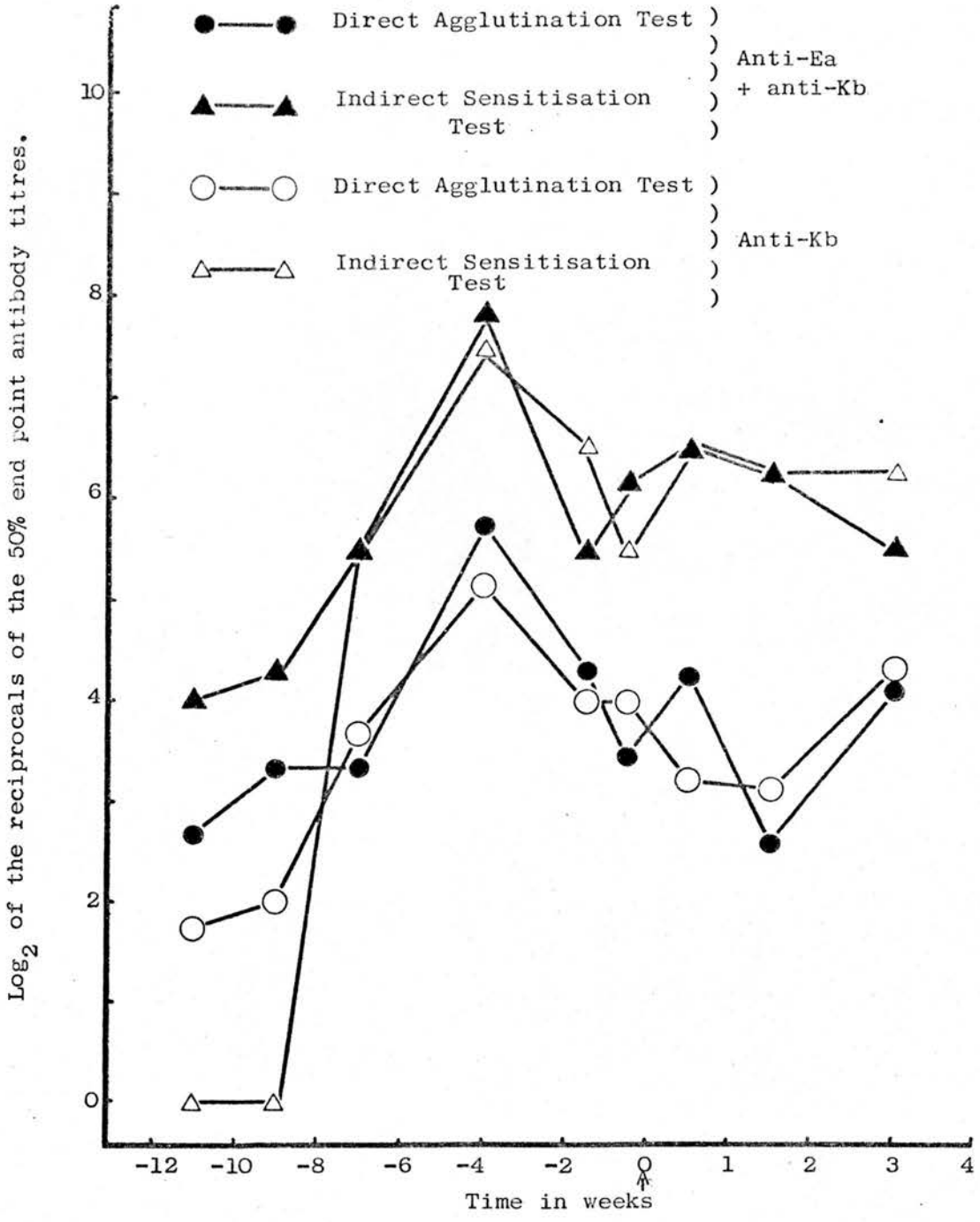


Fig. 1.5 Antibody titres in serial serum samples from sow 301 during pregnancy and after parturition to boar No. 8 (parturition at week 0), as monitored by red cells from No. 8 (anti-Ea + anti-Kb) and RW (anti-Kb).

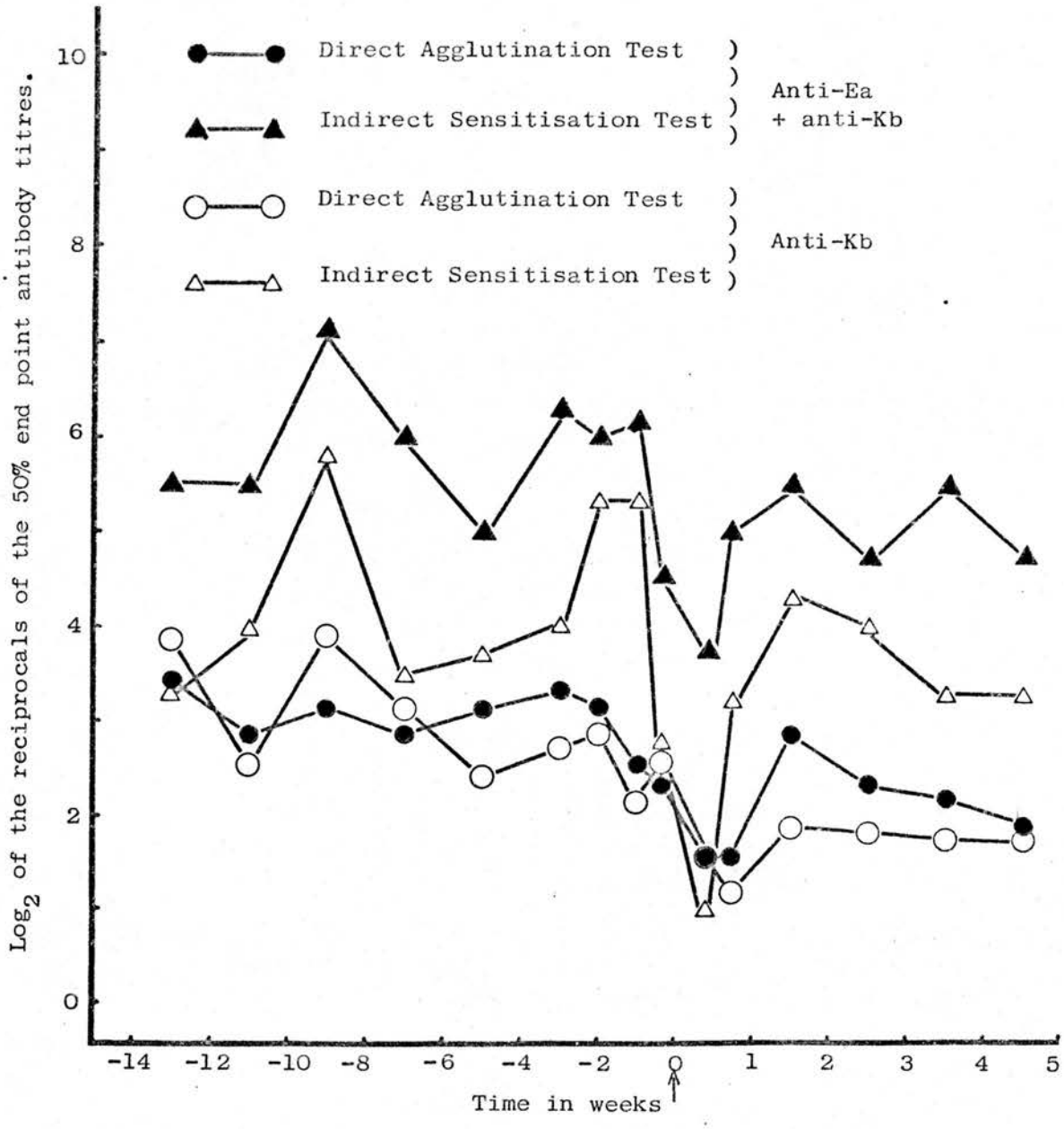


Fig. 1.6 Antibody titres in serial serum samples from sow 301 during pregnancy and after parturition to boar No. 312 (parturition at week 0) as monitored by red cells from No. 8 (anti-Ea + anti-Kb) and RW (anti-Kb).

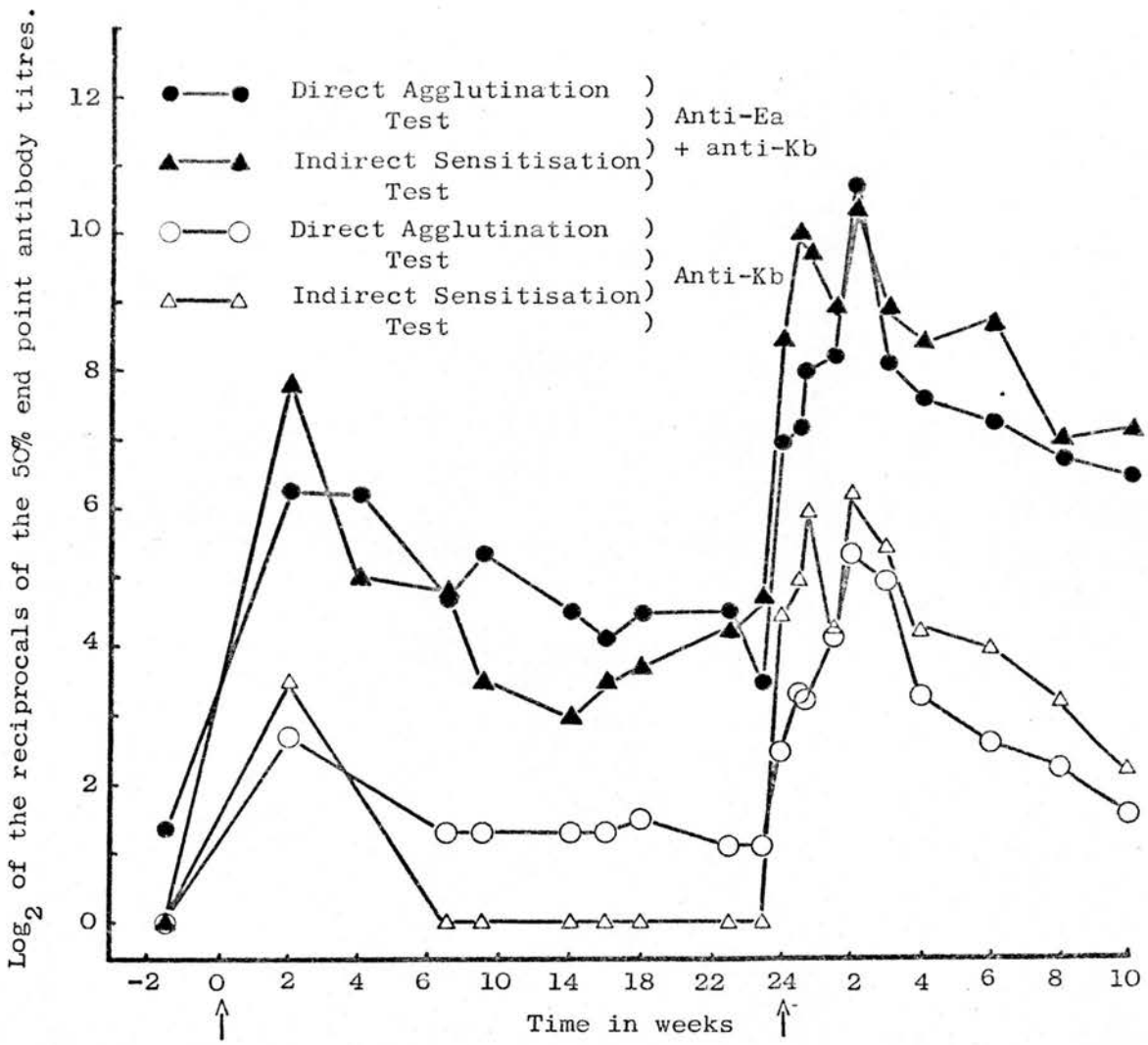


Fig. 1.7 Antibody titres in serial serum samples from sow 302 during pregnancy and after parturition to (1) an Ea positive, Kb positive boar (parturition at week 0) followed by (2) another Ea positive, Kb positive boar, BFM2 (parturition at week 24) as monitored by red cells from T38P12 (anti-Ea + anti-Kb) and RW (anti-Kb).

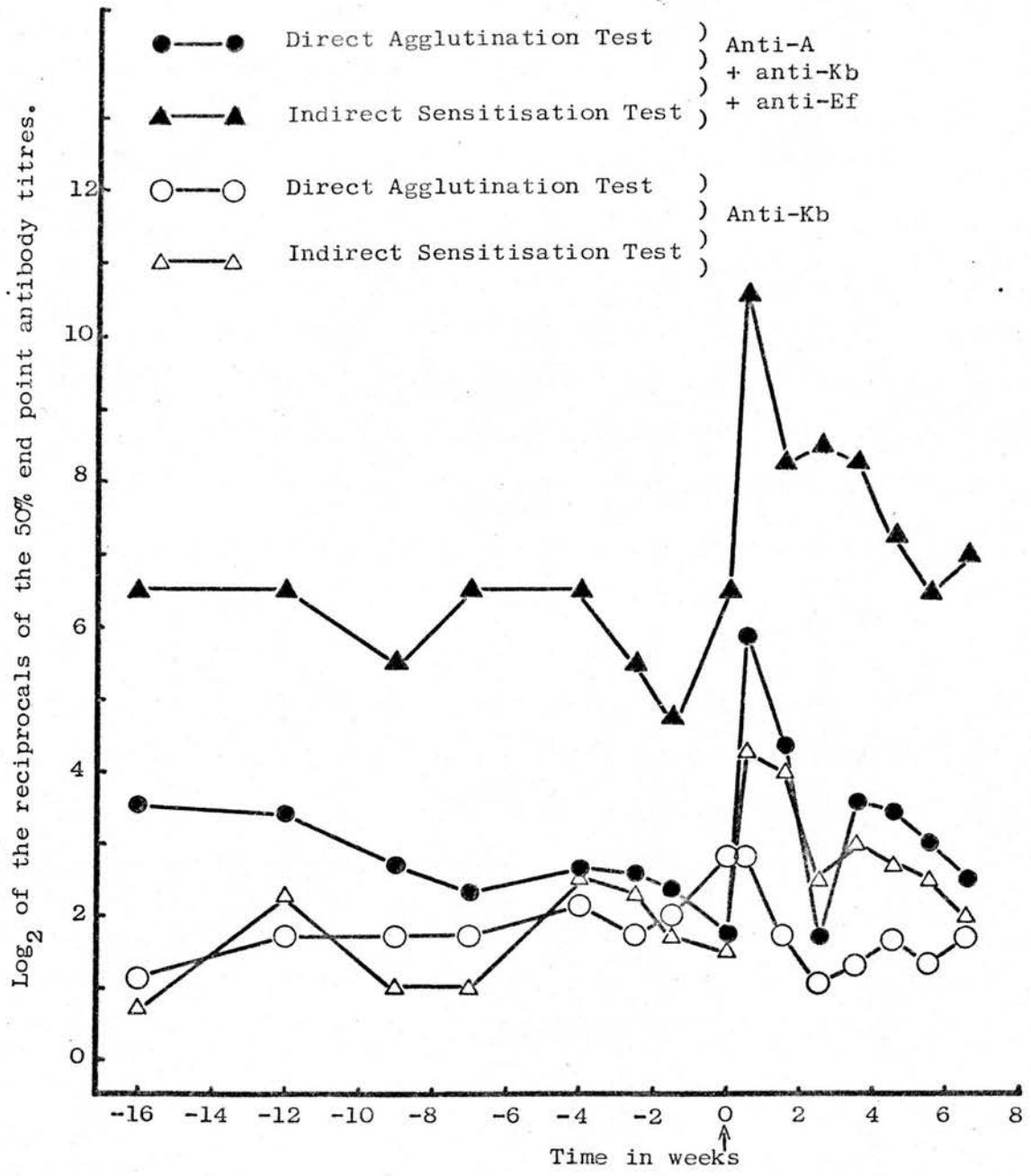


Fig. 1.8a Antibody titres in serial serum samples from sow 302 during pregnancy and after parturition to boar No. 311 (parturition at week 0) as monitored by red cells from No. 311 (anti-A + anti-Kb + anti-Ef) and RW (anti-Kb).

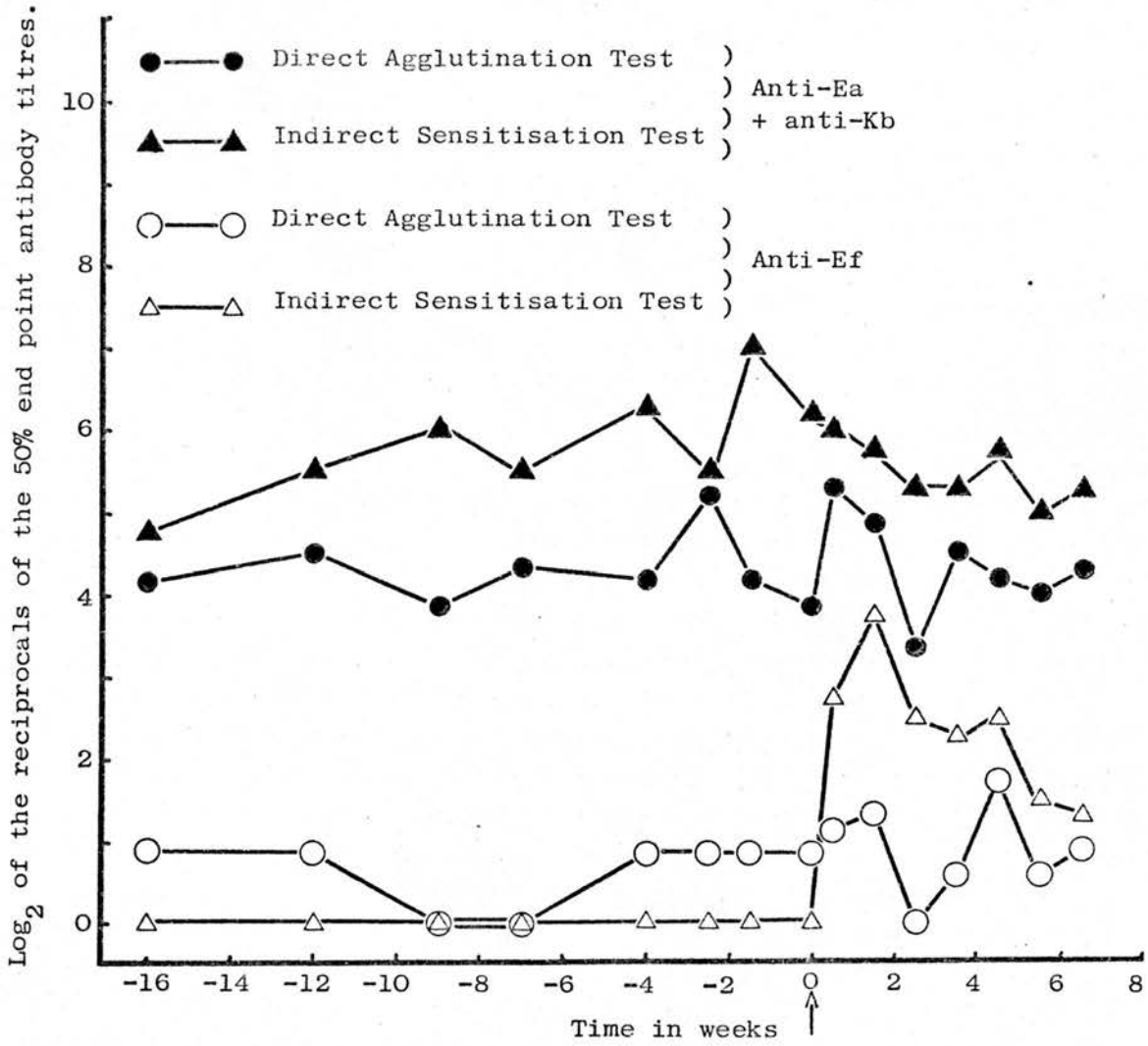


Fig. 1.8b Antibody titres in serial serum samples from sow 302 during pregnancy and after parturition to boar No. 311 (parturition at week 0) as monitored by red cells from No. 8 (anti-Ea + anti-Kb) and L412 (anti-Ef).

2. The incidence of iso-antibodies to red cell antigens in the sera of adult pigs

To assess the incidence of iso-antibodies to red cell antigens in the sera of adult pigs, one hundred and forty-seven samples taken from adult boars and sows and submitted to the Blood Group Research Unit for routine parentage checking were examined. These animals were bred pure and were the parents of piglets sent to the MLC performance testing stations, being mostly of the Large White and Landrace breeds with a few Welsh and British Saddlebacks.

Similarly, serum samples from twenty-four dams of clinical cases of thrombocytopenic purpura in piglets, received from the Veterinary Investigation Centres at Cambridge, Aberdeen and Edinburgh, were examined for red cell iso-antibodies. As it is considered that the thrombocytopenia in these animals is due to iso-immunisation of the sow by incompatible foetal thrombocytes *in utero* and subsequent absorption of the iso-antibodies from the colostrum by the piglets (Stormorken *et al.*, 1966; Lie, 1968; and Saunders and Kinch, 1968), it was decided to look at the sera of the dams of thrombocytopenic litters to see if red cell iso-antibodies had been produced.

Serum samples were tested in batches using the direct agglutination, haemolytic and indirect sensitisation (antiglobulin) techniques. Doubling dilutions of serum were tested against red cells from five animals of known red cell type in order to cover various combinations of all known types in the blood group panel. With the

cooperation of Hojny* this panel had been extended to cover fifty-four known red cell factors. Titres were recorded in the usual manner and the antibodies detected characterised by absorptions and checkerboard analyses. Reactions of less than fifty per cent avidity at the first dilution, i.e. where equal volumes of undiluted serum and red cell suspension were used, were ignored as it was not possible to analyse and confirm the presences of these by the absorption technique.

(a) The occurrence of iso-antibodies to red cell antigens in the sera of normal adult pigs

Of the samples examined, sixty-seven were taken from boars and eighty from sows. No antibodies apart from anti-A were detected by the haemolytic technique in the sera of either sex. Similarly, in the boars' sera, there was no evidence of antibodies other than anti-A by the direct agglutination and indirect sensitisation techniques (Table 2.I; Appendix II, Table 1).

On the other hand, of the sows' sera, fifty-nine (seventy-four per cent) either contained anti-A alone or were completely negative while twenty-one (twenty-six per cent) had other antibodies apart from anti-A (Table 2.I; Appendix II, Table 2). Analyses of these antibodies (Appendix II, Table 3) showed that anti-Ea and anti-Eb were far the most common (Table 2.2) and also that they were present at the highest levels (Table 2.2). Other antibodies present at low levels included anti-Ee, anti-Fa, anti-Ka, anti-Kb, anti-Kd and anti-La while in two of the

* Hojny, J., Czechoslovakian Academy of Sciences, Laboratory of Physiology and Genetics of Animals, Libechov, Czechoslovakia.

sera it was not possible to identify the antibodies present.

As crystal violet swine fever vaccine had been withdrawn in this country in August 1964, and all but one of the sows with iso-antibodies in their sera were born after this date (Appendix II, Table 4), this could be excluded as a possible cause of the production of the iso-antibodies. Further, most of these sows were young animals, thirteen of the twenty-one being with their first or second litters (Fig. 2.1), and all had been mated to boars of the same breed as themselves. Hence the chances of matings of incompatible red cell type occurring were less than if older cross-bred sows had been examined.

There was no apparent significant difference in incidence of iso-antibodies between the breeds of sows examined (Table 2.3). Neither was there a significant difference in the incidence of the individual iso-antibodies which were most common, anti-Ea and anti-Eb, between the breeds. However, the numbers of animals examined were too small, especially for the Welsh and British Saddleback breeds, to draw any definite conclusions as to breed incidence.

(b) <u>The occurrence of iso-antibodies to red cell antigens in the sera of dams of piglets affected with thrombocytopenic purpura</u>

Of twenty-four serum samples examined, twelve were negative for red cell iso-antibodies but seven of these contained the natural antibody, anti-A, alone. The other twelve showed evidence of red cell iso-antibodies six of

Table 2.1

Results of examining the sera of normal sows and boars for red cell antibodies.

Serum examination	Boars	Sows	Total
Anti A	51	47	98
Anti-A + iso-antibody	0	13	13
Iso-antibody	0	8	8
Negative	16	12	28
TOTAL	67	80	147

Table 2.2

Iso-antibodies identified in the sera of normal sows.

Iso-antibody	No. of times isolated	Titre Range (Log_2)	
		DA	IST
Anti Ea	4	1.29 - 3.71	1.20 - 5.50
Anti Eb	7	1.125 - 3.00	1.29 - 6.00
Anti Ea + anti La	1	3.71	5.50
Anti Ea + anti Ea	1	3.00	3.71
Anti Ea	1	1.33	-
Anti Ka	1	-	2.50
Anti Kb	1	1.33	-
Anti Kd	1	-	2.50
Anti Fa	2	1.125 - 1.71	2.29
Unidentified	2	1.00 - 1.125	1.00 - 1.125
TOTAL	21	1.00 - 3.71	1.00 - 6.00

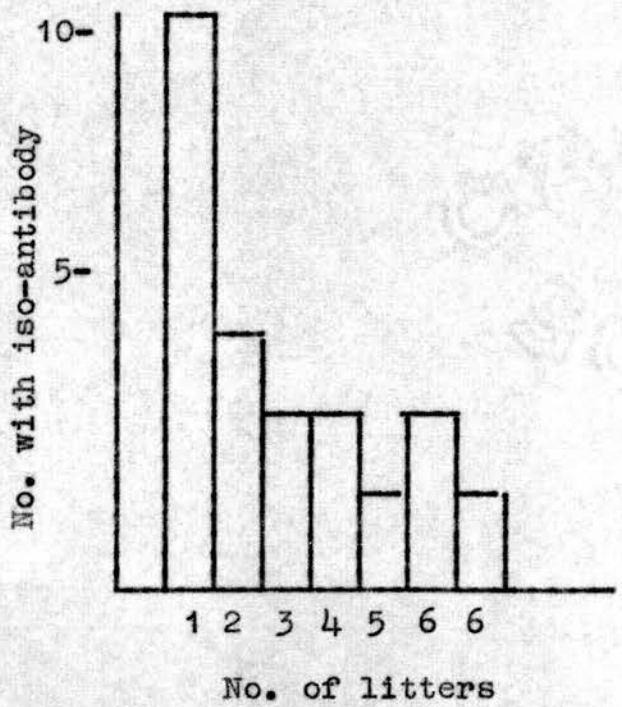
Table 2.3

Incidence of iso-antibodies according to breeds of sows examined.

Breed	No.examined	No. with iso-antibodies	No.with anti-Ea	No.with Anti-Eb
Large White	34	8	2	4
Landrace	36	10	3	3
Welsh	9	3	1	0
British Saddleback	1	0	0	0
TOTAL	80	21	6	7

Fig. 2.1

Frequency of iso-antibodies in relation to parity of the sow.



which had anti-A as well (Table 2.4). The iso-antibodies were all detected by means of the direct agglutination and indirect sensitisation techniques (Appendix II, Table 5) and identified by absorptions and checkerboard analyses (Appendix II, Table 6). No antibodies apart from anti-A were detected by the haemolytic technique.

Anti-Ea was the antibody most frequently isolated followed by anti-Eb (Table 2.5). These antibodies were also present at the highest titres. Other antibodies identified included anti-Ee, anti-Kb and anti-Lg; it was not possible to identify two of the antibodies present.

Vaccination with swine fever crystal violet vaccine could be precluded in all but two of these animals, one of which had a red cell iso-antibody present in its serum, the other of which had not.

In contrast to the sows in Results 2a, these sows fell into an older group, most having had at least three or four litters before sampling. Further, the majority had been bred with boars of a different breed from themselves. Most of the sows were of the Landrace or Welsh breeds and their crosses and had been mated to Large White boars.

Conclusions. These results further support the hypothesis that iso-immunisation by foetal red cells can occur in sows. Twenty-six per cent of the pure-bred sows and fifty per cent of those which had produced thrombocytopenic litters and which were mainly bred with boars of a different breed, had

Table 2.4

Results of examining the sera of sows which had produced litters suffering from thrombocytopenic purpura for red cell antibodies.

Serum examination	No. of animals
No antibody	5
Anti-A alone	7
Anti-A and iso-antibody	6
Iso-antibody alone	6
TOTAL	24

Table 2.5

Iso-antibodies identified in the sera of sows which had produced litters suffering from thrombocytopenic purpura.

Iso-antibody	No. of times isolated	Titre Range (Log_2)	
		DA	IST
Anti Ea	7	1.33 - 3.67	2.50 - 5.00
Anti Eb	3	4.57 - 6.00	2.50 - 6.50
Anti Ee	1	1.71	1.50
Anti Kb	1	1.875	-
Anti Lg	1	-	5.50
Unidentified	2	1.125	1.71
TOTAL	15	1.125 - 6.00	1.50 - 6.50

red cell iso-antibodies present in their sera. The former were mostly young sows which had produced only one or two litters while the latter were older sows of third or fourth parity. Meanwhile no red cell iso-antibodies were found in the sera of sixty-seven comparable adult boars while the incidence of anti-A was similar in both sexes.

That red cell iso-antibodies were not present in all cases which had produced thrombocytopenic litters might merely be due to the fact that, while the thrombocytes of the foetuses carried antigens which were incompatible with the dam, their red cells did not necessarily always have antigens which were incompatible.

As blood sampling was carried out in all sows during lactation, it was at a favourable time for the detection of iso-antibodies produced following stimulation by foetal red cell antigens at parturition or during late pregnancy (Results 1).

Anti-Ea and anti-Eb were the iso-antibodies most frequently present in both groups of sows with several other iso-antibodies being present but mostly at low levels. The latter included anti-Ee, anti-Fa, anti-Ka, anti-Kb, anti-Kd, anti-La and anti-Lg.

3. The effect of maternal red cell iso-antibodies on the red cells of piglets

To investigate the effect of maternal iso-antibodies on the red cells of piglets, the litters of four sows were studied. The four sows all had anti-Ea in their sera and were all mated to a boar which was heterozygous for the Ea factor in order that both Ea negative and Ea positive piglets might be present in the litters, the former to act as controls for the effect of the antibody on the red cells of the latter.

The sows 88, 405, 301 and 302 were all artificially inseminated with semen from the same boar Balhary Field Marshall 2, the first two being kept in the minimal disease piggery and the other two under normal commercial conditions. Red cell typing of the forty-nine piglets produced using the reagents of the Blood Group Research Unit and blood samples taken before they received colostrum, showed that thirty-four were Ea positive and fifteen Ea negative (Appendix I, Tables 1c, 2c, 3c and 7c). Thirty-six piglets survived until the end of the period of study, there being eight, seven, four and seven Ea positive and two, two, four and two Ea negative animals respectively in the four litters. Statistical analyses was carried out on the results of the samples taken from these.

Post mortem examinations were carried out on all piglets which died and these confirmed stillbirth or death due to crushing within two days of birth in eleven cases. Of the other two, one was crushed at two weeks of age and the other

died from enteritis at the same age. In none of these could the effects of the anti-Ea be implicated as a cause of death.

The direct Coomb's test was carried out on all samples (Table 3.1) to detect any coating of the piglets' red cells by anti-Ea absorbed in their dams' colostrum. All precolostral samples were negative as were all samples from the Ea negative piglets. By twenty-four to thirty-six hours after ingesting colostrum for the first time, the red cells of all Ea positive piglets except three in the litter ex 405 gave positive results confirming that anti-Ea had been ingested and absorbed by them. By the fourth day, eleven of these were still giving positive reactions, mainly those in litters ex 88 and 302, but, by the seventh day, all but one piglet in litter ex 302 were negative.

The levels of anti-Ea in the serum of the piglets were followed using cells from T38P12 by the direct agglutination and indirect sensitisation techniques. All precolostral samples and all samples from Ea positive piglets were negative. Anti-Ea was therefore only detected in piglets which were Ea negative. In the litters of 88 and 405 only very low levels were present up to four days of age. In the litters of 301 and 302, however, relatively high levels were detected after receiving colostrum (Table 3.2). These were maintained for some time before showing a drop at about three to four weeks of age, and eventually gradually falling to very low levels at eight to ten weeks. After this, the antibody could be detected in some animals, especially by the

direct agglutination technique, but only at very low levels, up to fifteen weeks of age when the observations ceased.

When the anti-Ea levels in the sows' sera at parturition are considered (Table 3.3), it can be seen that 301 and 302 had much higher titres than 88 and 405. As might have been expected, the antibody levels were higher than and persisted longer in the sera of the offspring of the former than those of the latter.

Red cell counts, packed cell volumes and haemoglobin contents were carried out on the first six bleeds from the four litters (Appendix III, Table 1). Analyses of variance were carried out with degrees of freedom and sums of squares partitioned as in the following example for the haemoglobin content:-

Analysis of variance for haemoglobin data.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F value	Significance
(+ v -)	1	0.99	0.99	0.82	N.S.
Litters	3	24.49	8.16	6.74	XX
Bleeds	5	672.74	134.54	111.19	XXX
Litter X (+ v -)	3	0.00	0.00	0.00	N.S.
Litter X Bleeds	15	100.58	6.71	5.55	XX
Bleeds X (+ v -)	5	1.25	0.75	0.21	N.S.
Litter X Bleeds X (+ v -)	15	27.76	1.85	1.53	N.S.
Total within	168	202.91	1.21	-	-
Total	215	1030.72	-	-	-

N.S. - not significant
XX - significant at the 1 per cent level
XXX - significant at the 0.1 per cent level

Table 3.1

No. of piglets showing coating of antibody on their red cells by means of the direct Coomb's test.

Age	Litter ex Sow No.							
	88		405		301		302	
	Ea+(8)	Ea-(2)	Ea+(7)	Ea-(2)	Ea+(4)	Ea-(4)	Ea+(7)	Ea-(2)
Precolostral	0	0	0	0	0	0	0	0
24 - 36 h	8	0	4	0	4	0	7	0
4 d	4	0	0	0	1	0	6	0
7 d	0	0	0	0	0	0	1	0
13 d	0	0	0	0	0	0	0	0
20 d	0	0	0	0	0	0	0	0

Table 3.2

Log_2 of the 50% end point titres of anti-Ea in the sera of piglets after ingesting colostrum.

Age	Litter ex 301								Litter ex 302			
	321		322		324		327		335		339	
	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
Precolostral	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24 - 36 h	4.875	7.29	4.43	7.50	3.33	6.50	1.63	5.50	2.50	5.71	4.00	5.71
4 d	3.17	5.50	2.67	5.50	3.11	4.86	2.33	4.50	3.33	4.50	3.40	6.00
7 d	3.67	6.29	2.75	4.60	2.60	5.00	1.67	4.50	2.33	4.71	3.17	6.00
13 d	1.00	4.71	1.80	4.29	1.50	4.00	0.67	3.50	2.14	4.29	1.50	5.00
20 d	0.00	2.50	0.00	2.71	0.00	1.50	0.875	3.00	1.50	2.71	2.83	4.00
4 w	1.50	2.71	0.875	1.50	1.00	2.00	0.875	2.29	0.67	2.00	1.50	4.00
5 w	0.67	2.50	1.125	1.50	0.67	2.00	1.125	1.50	1.50	2.50	1.71	4.29
6 w	1.50	2.00	1.50	1.29	1.33	1.50	1.50	1.50	1.29	1.50	1.29	2.71
7 w	1.33	2.50	1.33	0.00	1.125	1.50	0.875	1.29	1.33	1.50	1.71	3.29
8 w	1.125	1.71	0.67	1.50	0.67	1.29	0.00	1.50	1.33	1.71	1.33	2.50
9 w	0.67	1.00	0.67	1.50	0.00	1.50	0.00	1.00	0.875	1.71	0.67	3.00
10 w	0.875	1.80	0.875	1.00	0.00	1.29	0.67	0.00	0.875	1.00	0.67	2.00
11 w	0.875	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.875	1.50	1.125	1.71
12 w	1.20	1.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.29
13 w	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.875	0.00	0.67	0.71
14 w	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.875	1.00
15 w	0.875	0.00	0.875	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.875	0.00

Table 3.3

Levels of anti-Ea in the sows' sera at parturition.

Sow No.	Log_2 of the 50% end point titres	
	DA	IST
88	2.00	1.50
405	1.50	0.71
301	3.25	5.00
302	7.00	8.50

The main effect of Ea positive versus Ea negative and the interactions involving this comparison were all not significant. Litter differences were significant at one per cent. The effect of time of bleeding was very highly significant ($P < 0.001$). All values reached their lowest level at four days and gradually recovered to almost the precolostral levels by twenty days (Table 3.4).

Conclusions. Although the anti-Ea was absorbed from the colostrum by the piglets, it had little effect on their haematological picture, no differences between the values of the Ea positive and Ea negative piglets being noted.

In the Ea positive piglets all the anti-Ea was mopped up by their red cells and none was left in the serum. In the Ea negative ones, on the other hand, the anti-Ea persisted in the serum for up to fifteen weeks. Hence, the persistence of maternally derived antibody depended to a great extent on whether it came in contact with the respective antigen or not. The tailing off in titre in the Ea negative ones corresponded to an increase in body size with consequent dilution of the antibody as well as normal metabolism of the globulins.

Table 3.4

Mean values with standard errors of the haematological examinations of the piglets in four litters from birth to twenty days of age.

Age		Rbc x 10^6 per cm^3	PCV	Hb g%	No.
Precolostral	Ea+	5.79 ± 0.12	41.9 ± 0.76	13.1 ± 0.28	26
	Ea-	5.66 ± 0.20	41.7 ± 1.23	13.1 ± 0.50	10
	Total	5.75 ± 0.10	41.8 ± 0.65	13.1 ± 0.25	36
24 - 36 h	Ea+	4.28 ± 0.13	29.0 ± 0.85	8.8 ± 0.28	26
	Ea-	3.99 ± 0.28	27.8 ± 2.01	8.6 ± 0.69	10
	Total	4.20 ± 0.12	28.7 ± 0.82	8.7 ± 0.27	36
4 d	Ea+	3.62 ± 0.11	27.4 ± 0.70	8.1 ± 0.20	26
	Ea-	3.31 ± 0.22	27.2 ± 1.55	7.9 ± 0.43	10
	Total	3.54 ± 0.10	27.3 ± 0.65	8.1 ± 0.18	36
7 d	Ea+	4.35 ± 0.13	32.3 ± 0.56	10.2 ± 0.33	26
	Ea-	4.04 ± 0.16	31.3 ± 0.60	9.8 ± 0.44	10
	Total	4.27 ± 0.11	32.0 ± 0.44	10.1 ± 0.27	36
13 d	Ea+	4.94 ± 0.10	37.8 ± 0.38	10.7 ± 0.21	26
	Ea-	4.09 ± 0.14	37.7 ± 0.46	10.9 ± 0.27	10
	Total	4.87 ± 0.08	37.8 ± 0.30	10.8 ± 0.17	36
20 d	Ea+	5.65 ± 0.09	38.5 ± 0.41	12.2 ± 0.14	26
	Ea-	5.46 ± 0.11	38.0 ± 0.39	12.1 ± 0.21	10
	Total	5.60 ± 0.07	38.4 ± 0.44	12.2 ± 0.12	36

4. The investigation of serological techniques for the detection of porcine thrombocyte antigen/antibody reactions.

For this investigation three systems were used:

(a) Porcine thrombocytes and hetero-antibodies produced to them in rabbits.

(b) Bovine thrombocytes and hetero-antibodies produced to them in pigs.

(c) Porcine thrombocytes and iso-antibodies produced to them in other pigs.

In this way the porcine thrombocyte antigens and their antibodies could be studied in isolation and in conjunction and the tests standardised independently for each component. All three systems were not always used for each technique studied.

(i) Thrombo-agglutination. This was the only technique which had already been used by other investigators (Stormorken et al., 1963; Saunders et al., 1966). Initially, several sera from the dams of thrombocytopenic piglets and sera which had been produced by iso-immunisation of pigs were tested by this method using platelet suspensions from a panel of pigs. The results were unsatisfactory, not giving clearcut end point titres and the degree of reproducability being low. These are illustrated by results on immune serum from pig 312 using platelet suspensions from the donor pig, 318, and another pig, 313. The procedures adopted to try and rectify these shortcomings are also illustrated on this system.

When platelets from 318 were tested with a preimmunisation sample of 312's serum (Plate 4.1, 1 - 4) a certain amount of

aggregation took place especially at the lower dilutions. After the immunisation course with washed platelet suspensions from 318, serum from 312 strongly agglutinated the platelets from that animal, especially when used without dilution or only diluted 1 in 2 with triton-saline (Plate 4.1, 5 - 6). At higher dilutions, 1 in 4 or 1 in 8, it became difficult to differentiate between the aggregates produced here and those produced in the preimmunisation sample (Plate 4.1, 7 - 8 and 1 - 2). Similarly, after absorbing the antibodies from the immune serum with platelets from the donor, aggregation of the latter was still produced by the former on back-testing, especially at the lower dilutions (Plate 4.1, 9 - 12).

In all cases the saline control, i.e. suspension of platelets in triton-saline alone, was homogeneous (Plate 4.2, 1) while aggregates were produced even when 312's platelets were tested with its own serum (Plate 4.2, 2 - 4). When platelet suspensions from other animals, e.g. 313, were tested with the immune sample (Plate 4.2, 5 - 8) it became impossible to decide whether some reactions were positive or negative, even when photographs were available for direct comparison (Plates 4.1 and 4.2). Similarly, when the immune sample, absorbed with platelets from 318 was tested with platelets from another animal, 313, it was equally difficult to decide whether reactions were positive or negative (Plate 4.2, 9 - 12).

Therefore definite negative reactions, i.e. like the saline control (Plate 4.2, 1), and definite positives (Plate 4.1, 5 and 6) could easily be recognised but those of

intermediate avidity could not be classified.

To try and overcome this defect, the immune serum sample from 312 was fractionated by passing through a column of Sephadex G200 beads and eluted with 0.2 M sodium chloride, buffered at pH 8.0 with 0.1 M tris. The elution pattern showed the usual three main peaks, 19S, 7S and 4.5S, and the contents of the tubes corresponding to each of these were pooled, avoiding these where the peaks overlapped (Fig. 4.1). This gave a rough separation into a peak which contained mainly the IgM (19S), one which contained mainly the IgG (7S) and a third which contained mainly the albumin (4.5S) but which was contaminated with IgG and other serum proteins as shown by immuno-electrophoresis (Plate 4.3, 1 - 3).

After concentration to roughly the original volume, the fractions were tested against platelet suspensions from 318. The 7S fraction gave the strongest reactions (Plate 4.4, 5-8) similar to that given by the whole serum (Plate 4.1, 5 - 8). Aggregates were also produced by the 19S and 4.5S fractions (Plate 4.4, 1 - 4 and 9 - 12) and the end-points were still not clearcut. Similarly, the fractions when tested against platelets from the same animal, 312, still produced aggregates especially at the lower dilutions (Plate 4.5, 1 - 12) similar to that produced by the whole serum (Plate 4.2, 2 - 4).

Hence, attempts to get rid of the non-specific aggregation by fractionating the serum failed, the results produced being no better than with whole serum.

Similarly, running the tests at different temperatures, 4°C, 20°C and 37°C, had little effect on the non-specific

aggregation. The latter could, however, be reduced by increasing the EDTA present but this also tended to depress the specific agglutination and reduced and sometimes abolished the avidity of positive reactions.

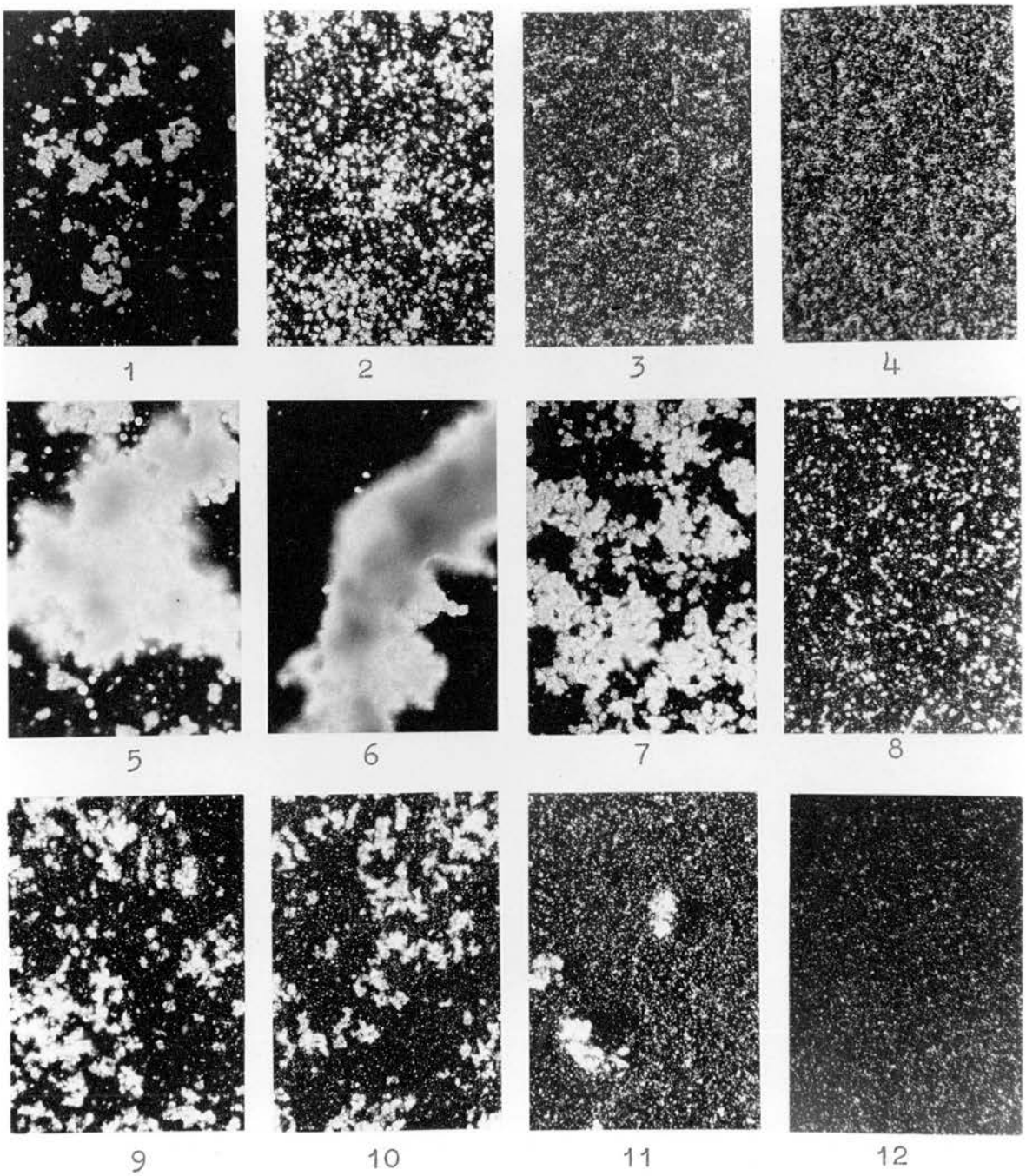
Plate 4.1 Thrombo-agglutination test x 250: serum from pig 312 (the recipient for the immunisation) and suspension of platelets from pig 318 (the donor).

Photographs 1-4. Preimmunisation sample of serum, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 5-8. Immune sample of serum, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 9-12. Immune sample of serum absorbed twice with platelets from 318, doubling dilutions from neat to 1 in 8 in triton-saline.

PLATE 4.1



1 2 3 4

5 6 7 8

9 10 11 12

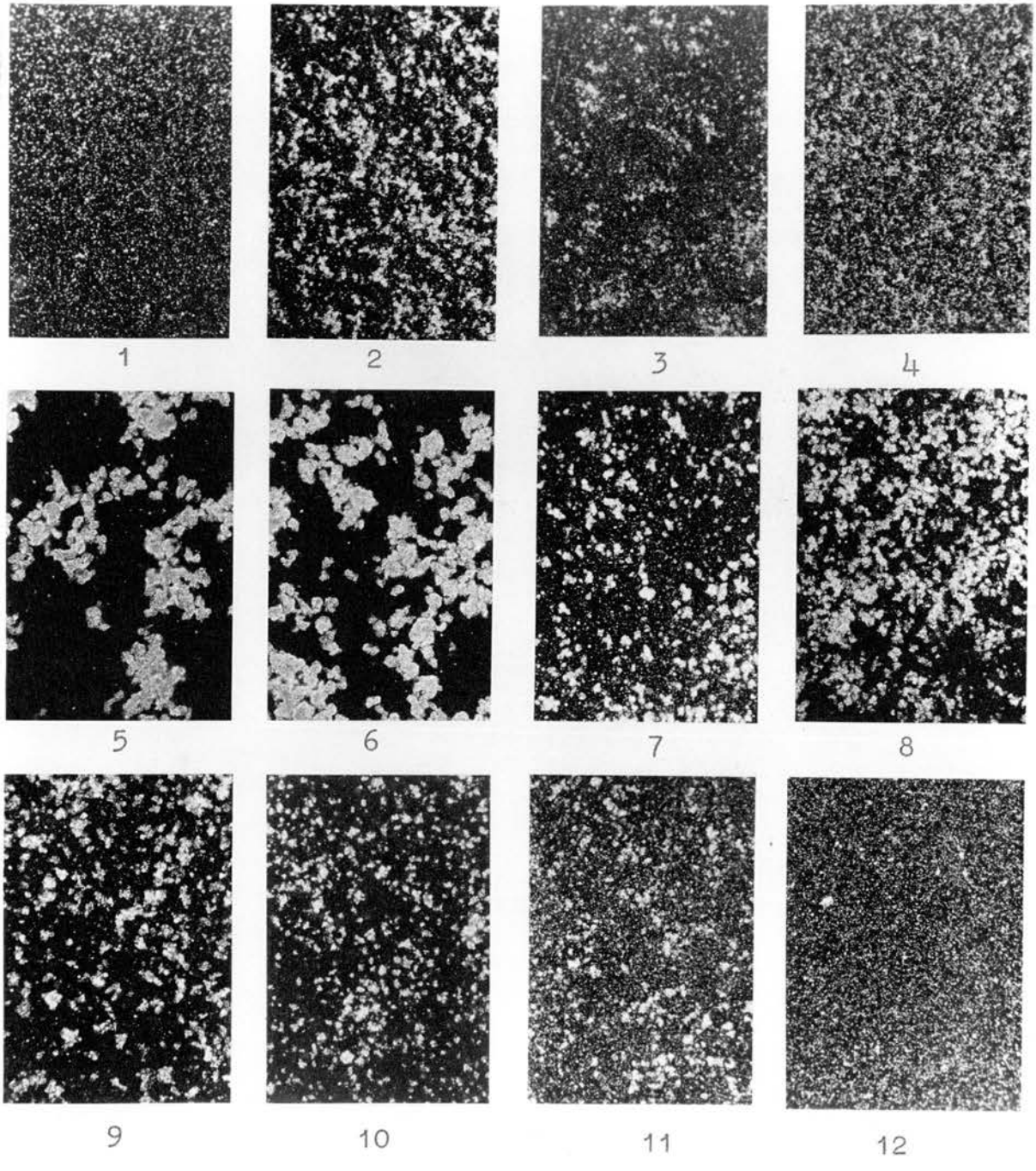
Plate 4.2 Thrombo-agglutination test x 250: serum from 312; platelets from pig 313 (litter-mate of 318).

Photographs 1-4. 1. Saline control; 2-4. Preimmunisation sample of serum, doubling dilutions from neat to 1 in 4 in triton-saline.

Photographs 5-8. Immune sample of serum, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 9-12. Immune sample of serum absorbed twice with platelets from 318, doubling dilutions from neat to 1 in 8 in triton-saline.

PLATE 4.2



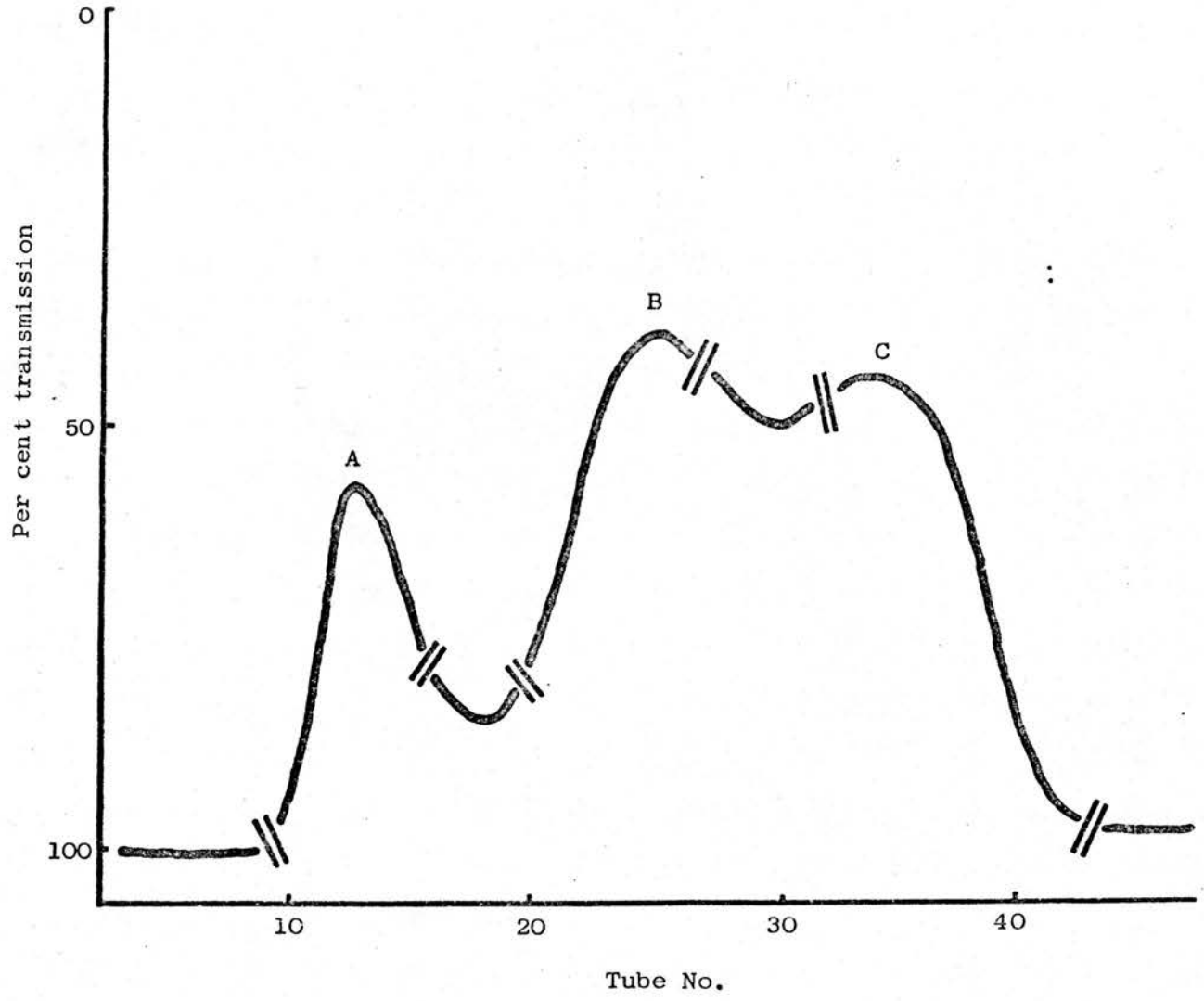


Fig. 4.1 Sephadex G 200 fractionation of immune serum from pig 312. Tubes under the three main peaks were pooled as follows:-

A (19S) - Tubes 10-15

B (7S) - Tubes 20-26

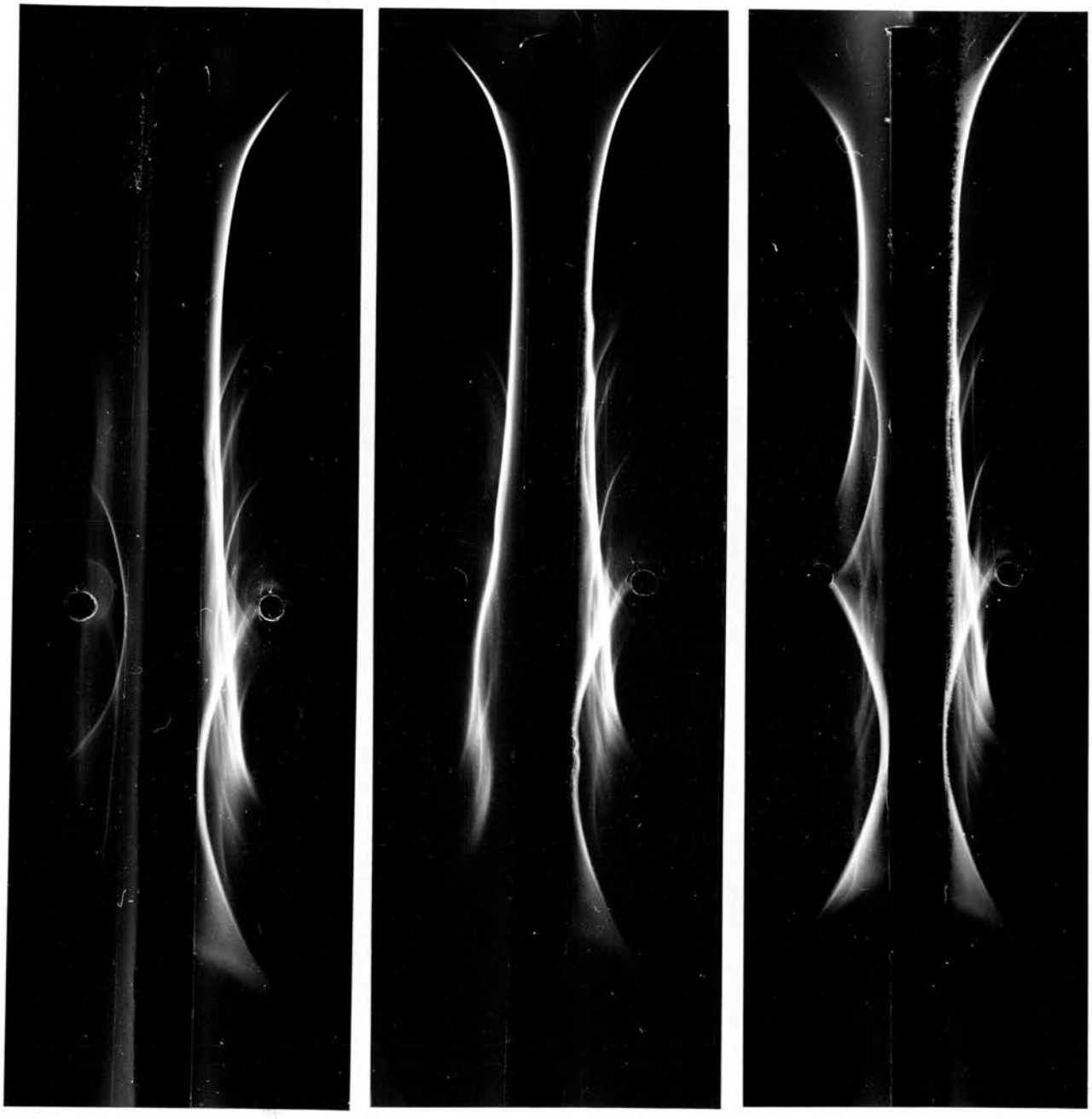
C (4.5S)- Tubes 31-41

Plate 4.3 Characterisation of pooled fractions after Sephadex G 200 fractionation of immune serum from pig 312 (Fig. 4.1); anode at left hand side, cathode at right hand side.

Photograph 1. 19S (peak A) fraction in upper well, whole porcine serum in lower well; rabbit anti pig globulin, R6, diluted 1 in 2 in phosphate buffered saline, pH 7.2, in trough.

Photograph 2. 7S (peak B) fraction in upper well, whole porcine serum in lower well; rabbit anti pig globulin, R6, diluted 1 in 2 in phosphate buffered saline, pH 7.2, in trough.

Photograph 3. 4.5S (peak C) fraction in upper well, whole porcine serum in lower well, rabbit anti pig globulin, R6, diluted 1 in 2 in phosphate buffered saline, pH 7.2, in trough.



1 2 3

PLATE 4.3

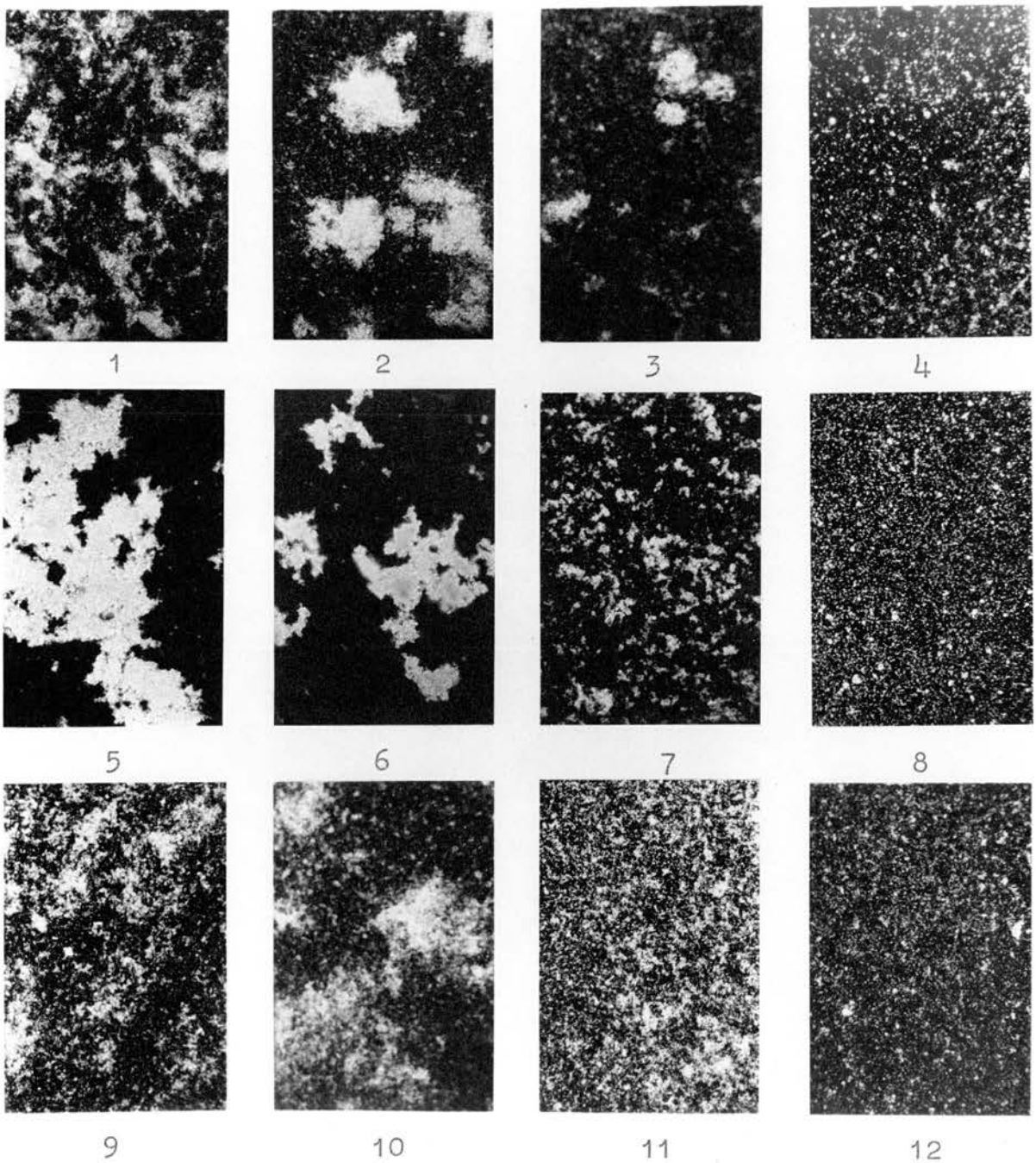
Plate 4.4 Thrombo-agglutination test x 250: pooled fractions of immune serum from 312 (Fig. 4.1 and Plate 4.3) tested against platelet suspension from 318.

Photographs 1-4. 19S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 5-8. 7S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 9-12. 4.5S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

PLATE 4.4



1 2 3 4

5 6 7 8

9 10 11 12

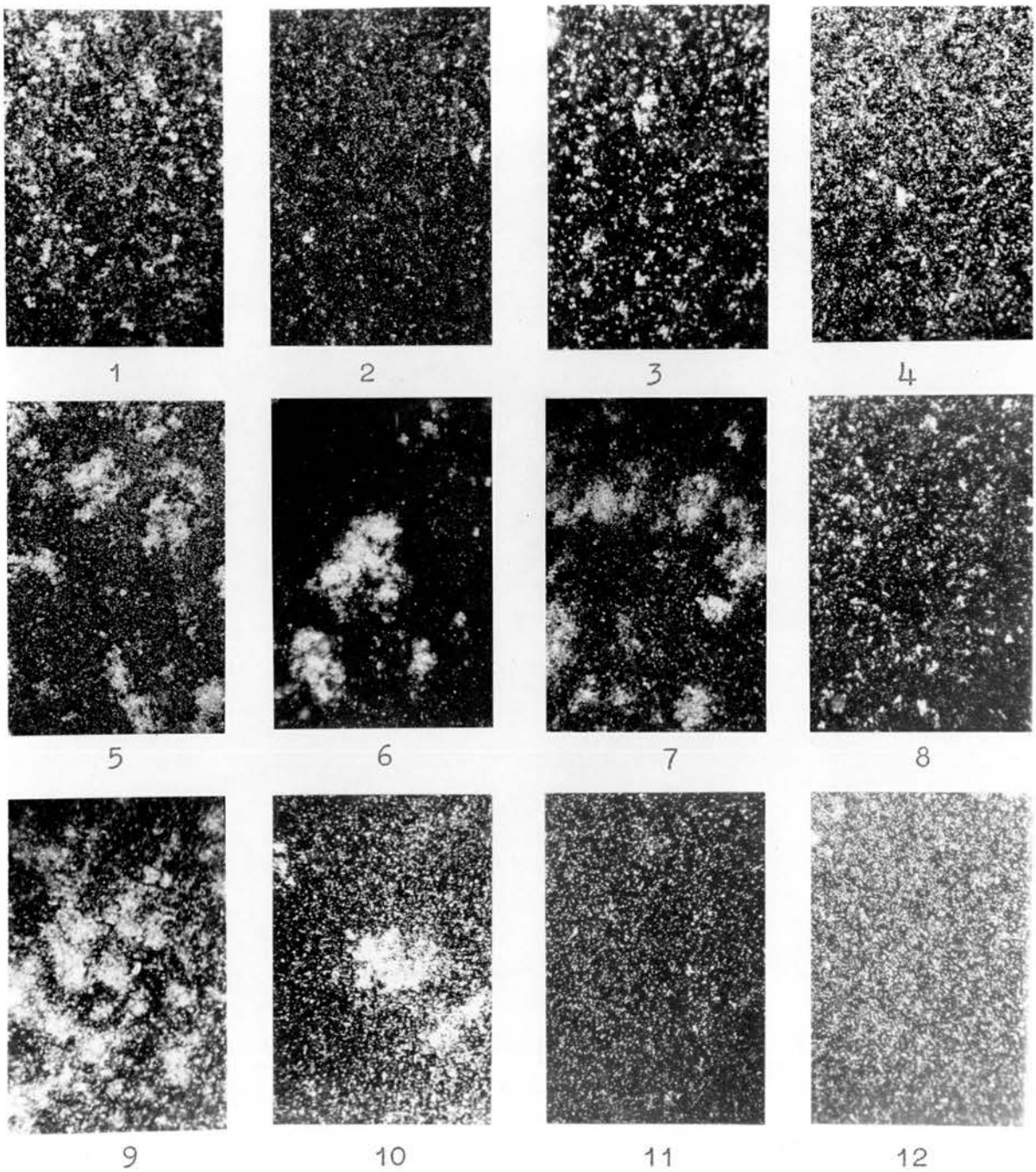
Plate 4.5 Thrombo-agglutination test x 250: pooled fractions of immune serum from 312 (Fig. 4.1 and Plate 4.3) with platelet suspensions from pig 312.

Photographs 1-4. 19S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 5-8. 7S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

Photographs 9-12. 4.5S fraction, doubling dilutions from neat to 1 in 8 in triton-saline.

PLATE 4.5



(ii) Fluorescent antibody technique. The porcine anti-bovine-platelet and rabbit anti porcine platelet sera were both conjugated with fluorescein isothiocyanate (FITC). When both were applied to smears of the respective platelets, bright fluorescence was observed. This fluorescence was found to be specific as the usual controls were negative:
(a) Pre-treatment of the smears with unconjugated antisera.
(b) Treatment of the smears with conjugated antisera which had previously been absorbed with the respective platelets.
(c) Treatment of the smears with a conjugated sample of preimmunisation serum.

Three isologous systems were then tested, i.e. where the porcine platelet smears were treated with their respective porcine iso-antibodies. All three sera tested had been found to contain iso-antibodies to porcine platelets by other techniques as follows:
(a) Serum from 312 which was known to contain iso-antibodies to platelets from pig 318 by means of the thrombo-agglutination technique (Results 4 (i)).
(b) Sera from 306 and 307 which were both known to contain iso-antibodies to platelets from pig 311 by means of the antiglobulin consumption test (Results 4 (v)).

All three sera were conjugated with FITC and applied directly to smears of the respective platelets. No fluorescence resulted. They were then further investigated by the indirect method, i.e. using a conjugated rabbit anti pig globulin serum after the specific porcine iso-antisera had been washed off. In all cases, the platelets fluoresced

non-specifically, i.e. they fluoresced with the conjugated antiglobulin whether they had previously been treated with the iso-antisera or not.

It was thought that this effect was due to the difficulty in washing platelets free of their own plasma proteins but washing the platelet suspensions for up to ten times in triton-saline before making the smears failed to get rid of this effect. Therefore this technique was abandoned.

(iii) Immuno-diffusion in agar gel. On immuno-diffusion in agar gel precipitin lines were produced between the rabbit anti porcine platelet serum and a suspension of sonicated porcine platelets (Plate 4.6). Reactions were observed up to a 1 in 8 dilution of the immune serum and to a 1 in 4 dilution of the platelet suspension and no precipitation occurred between the pre-immunisation sample of rabbit serum and the porcine platelet suspension.

On immuno-electrophoresis, it was seen that the precipitin lines occurred in the Ig G_2 region of the rabbit serum (Plate 4.7).

When the rabbit anti porcine platelet serum was tested against porcine plasma and porcine serum (Plate 4.8), precipitin lines were still produced especially with the plasma. As these were both free of platelets, antibodies had been produced to plasma proteins, especially fibrinogen, absorbed by the platelets and released by the sonication.

When the immune serum was absorbed with pig plasma and tested back against the porcine platelet suspension, no

precipitin lines were produced and no specific lines could therefore be detected to the antigens of the platelets themselves by this technique.

The pig anti bovine platelet serum gave a similar picture (Plate 4.9). When tested against the bovine platelet suspension, sharp precipitin lines were produced at dilutions of serum out to 1 in 8. Similarly, precipitin lines were seen against bovine plasma with faint ones seen against bovine serum. Again absorption with bovine plasma abolished the lines against the bovine platelet suspension and it seemed that the antibodies produced were directed mainly at fibrinogen which is closely associated with the platelets.

The same three porcine platelet/iso-antibody systems as investigated in Results 4 (ii) were also tried by this technique and, as no precipitin lines were apparent, this technique was discarded.

Plate 4.6 Immuno-diffusion: sonicated porcine platelet suspensions in both centre wells, the upper one at a concentration of 300 x 10^3 per mm^3, lower one at a concentration of 150 x 10^3 per mm^3, both in phosphate buffered saline, pH 7.2.

The same serum samples in peripheral wells in both cases: in centre right is a preimmunisation sample of serum from rabbit 17, undiluted, while in bottom right is a similar sample diluted 1 in 2 in phosphate buffered saline, pH 7.2. The immune samples of serum from rabbit 17 go from the bottom left well to the top right one by doubling dilutions from neat to 1 in 8 in phosphate buffered saline, pH 7.2.

PLATE 4.6

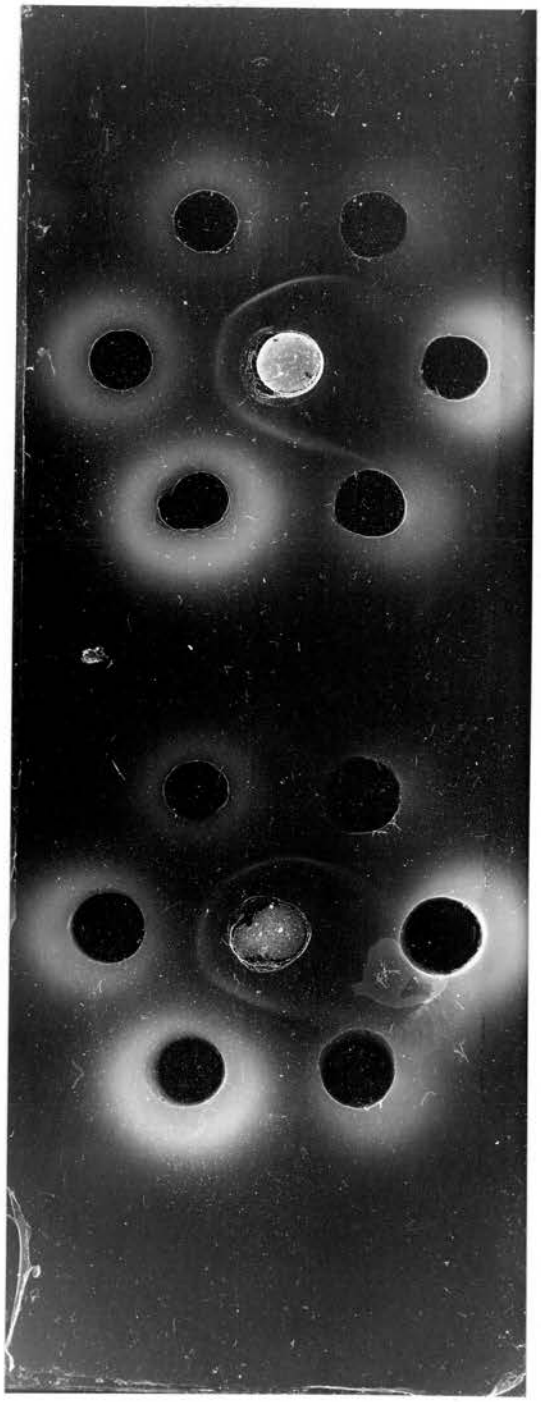


Plate 4.7 Immuno-electrophoresis: anode at left hand side, cathode at right hand side.

Upper well - immune serum from rabbit 17, neat.

Lower well - immune serum from rabbit 17, diluted 1 in 2 in phosphate buffered saline, pH 7.2.

Trough - suspension of sonicated pig platelets, 300 x 10^3 per mm^3 in phosphate buffered saline, pH 7.2.

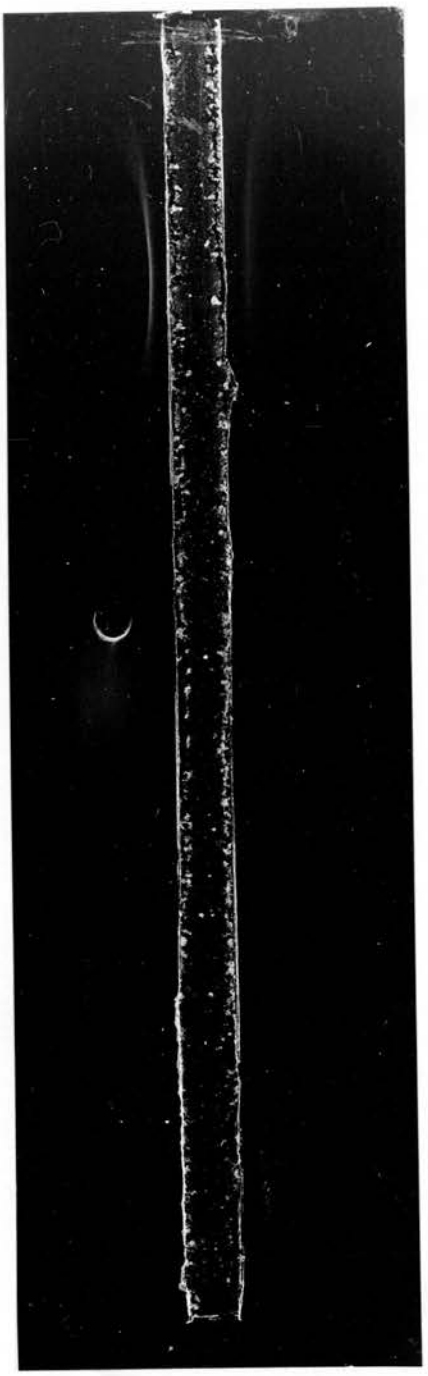


PLATE 4.7

Plate 4.8 Immuno-diffusion.

Photograph 1. Middle wells, left to right contain porcine plasma, neat by doubling dilutions to 1 in 16 in phosphate buffered saline, pH 7.2; and then repeated.

Top wells, left to right contain preimmunisation sample of serum from rabbit 17, neat, diluted 1 in 2, neat, diluted 1 in 2, neat; followed by immune serum from rabbit 17 in a similar sequence of dilutions.

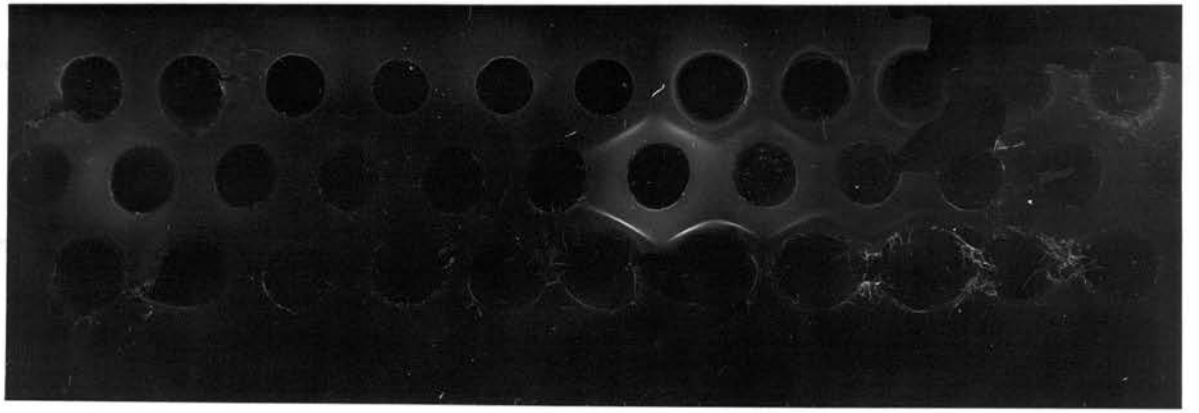
Bottom wells, left to right contain preimmunisation sample of serum from rabbit 17 diluted 1 in 8, 1in 4, 1 in 8, 1 in 4, 1 in 8; followed by a similar sequence of dilutions of immune serum from rabbit 17.

Photograph 2. Middle wells, left to right contain porcine serum, neat by doubling dilutions to 1 in 16 in phosphate buffered saline, pH 7.2; and then repeated.

Top and bottom wells contain the same samples as in photograph 1.

PLATE 4.8

1



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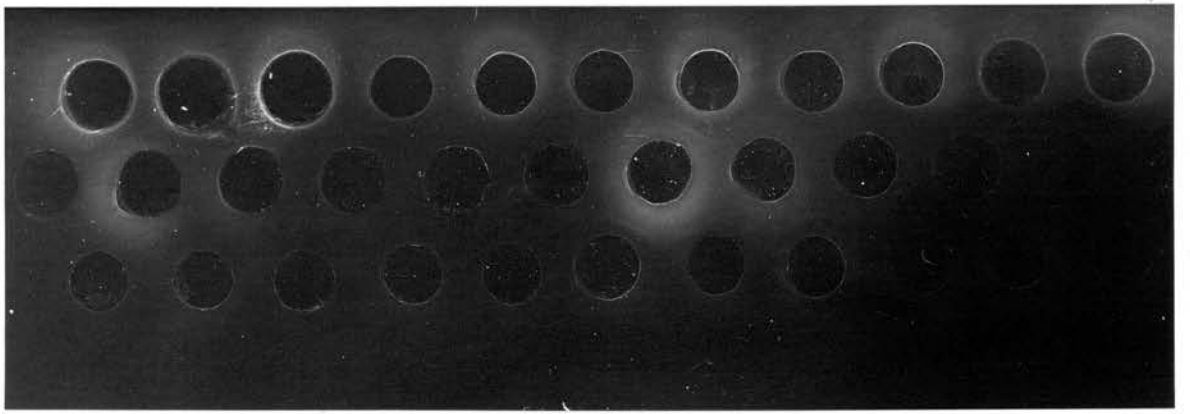


Plate 4.9 Immuno-diffusion.

Photograph 1. Middle wells, left to right contain a sonicated suspension of bovine platelets, 300 x 10^3 per mm^3 in phosphate buffered saline, pH 7.2, by doubling dilutions to 18.75 x 10^3 per mm^3; and then repeated.

Photograph 2. Middle wells, left to right contain bovine serum by doubling dilutions in phosphate buffered saline, pH 7.2, from neat to 1 in 16; and then repeated.

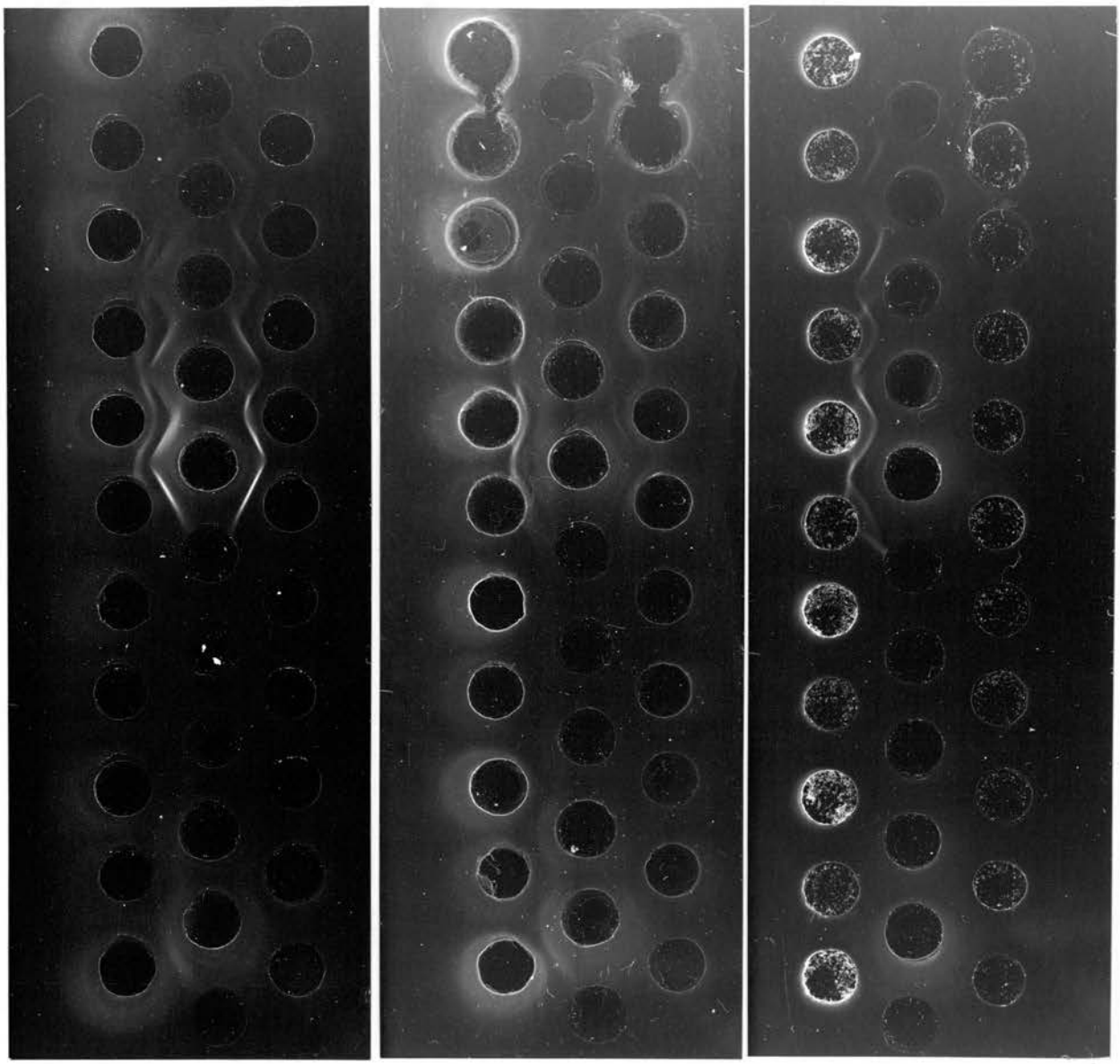
Photograph 3. Middle wells, left to right contain bovine plasma by doubling dilutions in phosphate buffered saline, pH 7.2, from neat to 1 in 16; and then repeated.

Top and bottom wells follow a similar pattern for all three photographs:

Top wells contain preimmunisation samples of serum from pig 228, neat, diluted 1 in 2, neat, diluted 1 in 2, neat; followed by immune serum from pig 228 in a similar sequence of dilutions.

Bottom wells contain preimmunisation samples of serum from pig 228, diluted 1 in 8, 1 in 4, 1 in 8, 1 in 4, 1 in 8; followed by immune serum from pig 228 in a similar sequence of dilutions.

PLATE 4.9



1 2 3

(iv) Complement fixation test. All three systems were used for investigating this technique, the rabbit anti porcine platelet serum being used to standardise the test for the antigen (porcine platelets) while bovine platelets were then used to standardise the test for porcine sera (with the porcine anti bovine platelet serum).

Following on Results 4 (iii) both the rabbit anti porcine platelet and pig anti bovine platelet sera had to be absorbed with the respective plasmas until no more precipitation was produced to get rid of the antibodies to plasma proteins in each which would give false reactions.

Initially in the test system the haemolysin was titrated (Appendix 4, Table 1) and at least two 100% haemolytic units used throughout (i.e. a dilution of 1 in 1000). The complement was then titrated (Appendix 4, Table 2) and five 50% units (i.e. a dilution of 1 in 80) used for the complement fixation test. Using a suspension of 300 x 10^3 porcine platelets per mm^3 the titre of the immune rabbit serum, R17, was 1 in 64, all controls being satisfactory (Appendix 4, Table 3).

To find the optimal concentration of porcine platelets which gave the highest titre in the rabbit serum, a checkerboard titration was set up (Appendix 4, Table 4). Taking a 50% haemolysis end point a concentration of 150,000 per mm^3 gave a titre of 1 in 128 (Table 4.1) and this was the concentration used for further investigations of this technique.

The serum from pig 228 was then examined for antibodies

to bovine platelets. Initially preimmunisation and immune samples of the serum were heat inactivated at 56°C and tested for fixation of guinea pig complement (three 50% haemolytic units) at three different temperatures. Fixation at 4°C overnight was most effective (Appendix 4, Table 5) and showed a titre of 1 in 80 after the prozone effect due to the procomplementary effect of inactivated porcine serum. Fixation at 20°C (Appendix 4, Table 6) and 37°C (Appendix 4, Table 7) were less efficient, especially the latter. The controls showed the procomplementary effects of the serum to be present up to a dilution of 1 in 20 in each case.

A similar routine was carried out with pig serum which had been inactivated both by heating and by treatment with formalin (Appendix 4, Tables 8-10). In this case five 50% haemolytic units of guinea pig complement were used and fixation was again observed, especially after overnight incubation at 4°C but to a lower titre (1 in 20) than in the previous case. The serum had, however, become anti-complementary as seen by the controls using two and one 50% haemolytic units of complement. With the porcine serum, therefore, the highest titre was achieved with three 50% haemolytic units of guinea pig complement and overnight fixation at 4°C with serum which had been inactivated by heating alone (Table 4.2).

From these experiments it was decided to test the isologous systems using a platelet suspension of 150,000 per mm^3 and porcine sera which had been inactivated by

heating only, with three 50% units of guinea pig complement and overnight fixation at 4°C. Serum samples from 306, 307 and 312 were tested with platelets suspensions from the respective donors for the immunisation, both preimmunisation and immune samples being used in each case (Tables 4.3 - 4.5). In none of these systems was there any evidence of fixation of the guinea pig complement. All three sera showed a procomplementary effect up to dilutions of 1 in 10 to 1 in 20, 100% haemolysis of the indicator system being produced at these and lower dilutions by one 50% haemolytic unit of guinea pig complement.

Table 4.1

Complement Fixation Test: Serum ex rabbit 17 and porcine platelets (150 x 10^3 per mm^3). Overnight fixation at 4°C.

Serum ex R17		Reciprocal dilutions of Serum								Antigen Controls		
		4	8	16	32	64	128	256	512			
Preimmunisation		4	4	4	4	4	4	4	4			
Immune		0	0	0	0	0	2	4	4			
Serum Controls	5uC[1]	4	4	4	4	4	4	4	4	4	4	4
	2uC[1]	4	4	4	4	4	4	4	4	4	4	4
	1uC[1]	2	2	2	2	2	2	2	2	2	2	2

Table 4.2

Complement Fixation Test: Serum ex pig 228 and bovine platelets (150 x 10^3 per mm^3). Overnight fixation at 4°C. Serum inactivated by heating only.

Serum ex 228		Reciprocal dilutions of Serum								Antigen Controls		
		5	10	20	40	80	160	320	640			
Preimmunisation		4	4	4	4	4	4	4	4			
Immune		4	4	0	0	2	4	4	4			
Serum Controls	3uC[1]	4	4	4	4	4	4	4	4	4	4	4
	2uC[1]	4	4	4	4	4	4	4	4	4	4	4
	1uC[1]	4	4	3	2	2	2	2	2	2	2	2

Table 4.3

Complement Fixation Test: Serum ex 306; platelets ex 311 (150×10^3 per mm^3). Overnight fixation at 4^oC.

Serum ex 306	Reciprocal dilutions of serum								Antigen Controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Immune	4	4	4	4	4	4	4	4			
Serum Controls 3uC^1	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 2uC^1	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 1uC^1	4	4	3	2	2	2	2	2	2	2	2

Table 4.4

Complement Fixation Test: Serum ex 307; platelets ex 311 (150×10^3 per mm^3). Overnight fixation at 4^oC.

Serum ex 307	Reciprocal dilutions of serum								Antigen Controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Immune	4	4	4	4	4	4	4	4			
Serum Controls 3uC^1	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 2uC^1	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 1uC^1	4	4	2	2	2	2	2	2	2	2	2

Table 4.5

Complement Fixation Test: Serum ex 312; platelets ex 318 (150×10^3 per mm^3). Overnight fixation at 4^oC.

Serum ex 312	Reciprocal dilutions of serum								Antigen Controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Immune	4	4	4	4	4	4	4	4			
Serum Controls 3uC[1]	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 2uC[1]	4	4	4	4	4	4	4	4	4	4	4
Serum Controls 1uC[1]	4	4	4	2	2	2	2	2	2	2	2

(v) Antiglobulin Consumption Test. Two samples of rabbit anti pig globulin sera, R1 and R6, produced to whole pig serum and which contained antibodies to a wide spectrum of the components of normal pig serum as shown by immuno-electrophoresis (Plate 4.10) and two samples, R29 and R30, produced by injecting rabbits with their own red cells which had been sensitised with heterophile antibodies occurring in porcine sera and which contained antibodies primarily to the immunoglobulin components of porcine sera (Plate 4.11) were prepared.

Titrations of the anti pig globulin levels in these sera were carried out using pig red cells sensitised with one of the red cell reagents used for red cell typing by the indirect sensitisation technique. R6 and R29 were found to have the highest titres and they were selected for further use in the antiglobulin consumption test (Appendix 4, Table 11).

Four similar red cell reagents along with porcine red cells carrying the corresponding factors (Table 4.6) were titrated in checkerboard fashion with these two antiglobulin sera to find the optimal dilutions of both the reagents and the antiglobulins to use in the test (Table 4.7; Appendix 4, Table 12a, b, c and 13a, b and c).

A preliminary antiglobulin consumption was then set up using 0.5 ml serum from pig 306 and 1 x 10^9 platelets from pig 311 with red cells from L412 sensitised with reagent 30 as indicator for the titration of the antiglobulin, R6. The platelets, when mixed with the immune serum, completely removed the anti globulin titre while the preimmunisation

sample reduced the titre by only two dilutions (Table 4.8). Varying volumes of serum and numbers of platelets were then tested to ascertain the optimal quantities of each for the test (Table 4.9). As the volume of serum and the number of platelets were reduced, so the reduction of the anti-globulin titre became less until the positive reaction eventually disappeared with 0.25 ml of serum and 0.25×10^9 platelets. Conversely, as the volume of neat serum was increased, so the amount of non-specific reduction of the antiglobulin titre by the preimmunisation serum was increased, until, with 1 ml of neat serum, all the antiglobulin titre disappeared. From these, the optimal quantities for the test were fixed at 0.25 ml of neat serum and 0.5×10^9 platelets.

Immune serum from pig 306 was then fractionated by passing through a column of Sephadex G200 beads, elution being carried out with 0.2 M sodium chloride buffered at pH 8.0 with 0.1 M tris. The tubes of eluant in the 19S, 7S and 4.5S peaks were pooled missing out these where the peaks overlapped (Fig. 4.2) giving a rough separation with IgM, IgG and albumin rich fractions. After concentrating to the original volume, each was tested with platelets from pig 311 (Table 4.10). The 19S and 4.5S fractions and the platelet control all reduced the antiglobulin titre by one dilution while the 7S fraction almost completely removed it, thus confirming that the main antibody present was a 7S one.

Absorbing serum from 306 with red cells from 311 had no effect on the anti-platelet antibody (Table 4.11). However, the latter was removed by absorbing with platelets from 311,

two absorptions being required for the absorption to be complete. As the first part of the antiglobulin consumption test depended upon the adsorption of the anti platelet antibodies onto platelets and its subsequent detection, the absorption of antibodies from the serum could be tested for by retesting the supernatant serum which was normally discarded. Thus no special absorptions had to be carried out as this procedure was part of the technique.

The antiglobulin consumption test was also found to be effective in detecting antibodies to platelets from 311 in the immune serum from 307 using the same antiglobulin and the same indicator system. No antibodies could, however, be detected by this technique in serum from 312 with platelets from 318 despite the fact that antibodies could be demonstrated by the thrombo-agglutination technique (Results 4, (i)). All the indicator systems (Table 4.7) were tested using both the antiglobulin reagents without success.

Conclusions. Of the five techniques studied only two, thrombo-agglutination and antiglobulin consumption, detected iso-antibodies to porcine platelets, though both had their disadvantages. The former gave ill-defined end points, the interpretation of negative results being difficult, and was only good for positive reactions of strong avidity. The antiglobulin consumption technique gave consistent results, the differences between positive and negative reactions being clearcut, especially if a negative serum sample was incorporated for each platelet sample in each batch of tests

to detect non-specific uptake of antiglobulin by the platelets. However, apart from being laborious, this technique could not be used for measuring antibody titres in the sera and some iso-antibodies could not be detected with the indicator systems investigated.

Table 4.6

Components of the indicator systems studied.

Reagent	Factor	Cell Source	APG
30	Lg	308 ; L412	R6 ; R29
59	Gb	308 ; 8544	R6 ; R29
61	Ib	306	R29
75	Kb	308 ; 311 ; 191	R6

Table 4.7

Optimal dilutions of reagents and anti pig globulin sera for use in indicator systems.

Reagent		Anti pig globulin	
No.	Optimal Dilution	No.	Optimal dilution
30	1:50	R6	1:8
59	1:40	R6	1:8
75	1:20	R6	1:8
30	1:40	R29	1:16
59	1:40	R29	1:16
61	1:20	R29	1:16

Table 4.8

Antiglobulin consumption test: 0.5 ml serum ex 306; 1 x 10^9 platelets ex 311; indicator system reagent 30/red cells ex L412/APG R6.

	Reciprocal dilutions of APG										SC
	8	16	32	64	128	256	512	1024	2048	4096	
Serum ex 306 (Preimmune)	++	++	++	++	++	+	(+)	-	-	-	-
Serum ex 306 (Immune)	-	-	-	-	-	-	-	-	-	-	-
Platelet control	++	++	++	++	++	++	++	+	-	-	-
APG control	++	++	++	++	++	++	++	++	(+)	-	-

Table 4.9

Antiglobulin consumption test: Effect of varying volumes of serum ex 306 and numbers of platelets ex 311; indicator system reagent 30/red cells ex L412/APG R6.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Preimmunisation	-	-	-	-	-	-	-	-	-	-
Immune	-	-	-	-	-	-	-	-	-	-
Platelet Control	++	++	++	++	++	++	++	-	-	-
1 ml serum; 2×10^9 platelets										
Preimmunisation	++	++	++	++	++	+(+)	(+)	-	-	-
Immune	+(+)	+	-	-	-	-	-	-	-	-
Platelet Control	++	++	++	++	++	++	++	+	-	-
0.5 ml serum (diluted 1 in 2); 1×10^9 platelets										
Preimmunisation	++	++	++	++	++	++	+(+)	-	-	-
Immune	++	+	(+)	-	-	-	-	-	-	-
Platelet Control	++	++	++	++	++	++	++	++	-	-
0.25 ml serum; 0.5×10^9 platelets										
Preimmunisation	++	++	++	++	++	++	++	+	-	-
Immune	++	++	++	++	++	(+)	-	-	-	-
Platelet Control	++	++	++	++	++	++	++	+	-	-
0.25 ml serum (diluted 1 in 2); 0.5×10^9 platelets										
Preimmunisation	++	++	++	++	++	++	++	+	-	-
Immune	++	++	++	++	++	++	+	(+)	-	-
Platelet Control	++	++	++	++	++	++	++	++	-	-
0.25 ml serum (diluted 1 in 2); 0.25×10^9 platelets										

Table 4.10

Antiglobulin consumption test: testing fractions of immune serum ex pig 306 with platelets ex pig 311.

Fraction	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
19S	++	++	++	++	++	++	++	-	-	-
7S	++	+	-	-	-	-	-	-	-	-
4.5S	++	++	++	++	++	++	+(+)	-	-	-
Platelet Control	++	++	++	++	++	++	++	(+)	-	-
APG control	++	++	++	++	++	++	++	+(+)	-	-

Table 4.11

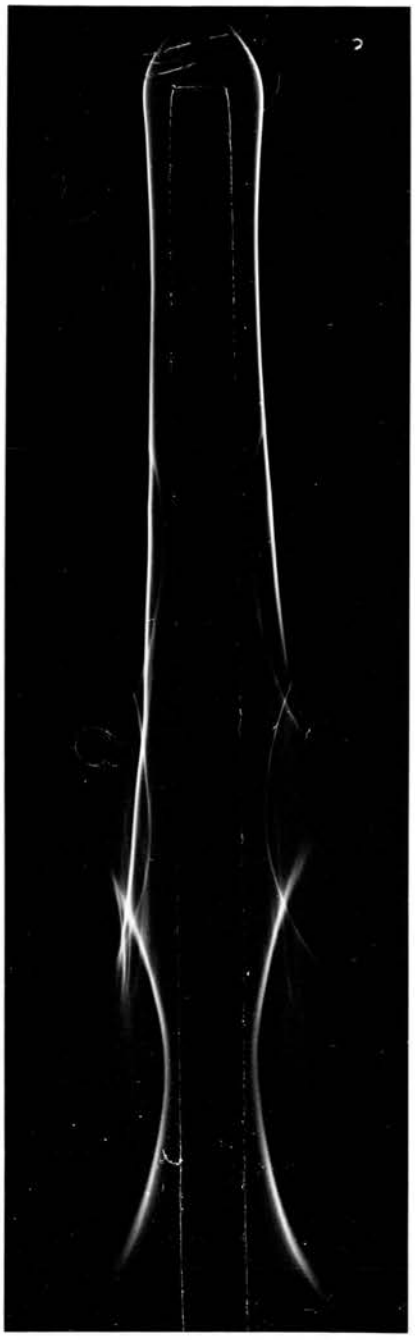
Antiglobulin consumption test: effect of absorbing immune serum ex pig 306 with 1. platelets ex pig 311 once, 2. platelets ex pig 311 twice, and 3. red cells ex pig 311 twice.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
Preimmunisation	++	++	++	++	++	++	++	(+)	-	-
Immune	++	+(+)	-	-	-	-	-	-	-	-
Immune absorbed 1	++	++	++	+(+)	(+)	-	-	-	-	-
Immune absorbed 2	++	++	++	++	++	++	+(+)	-	-	-
Immune absorbed 3	++	+	-	-	-	-	-	-	-	-
Platelet control	++	++	++	++	++	++	+(+)	-	-	-
APG control	++	++	++	++	++	++	++	+(+)	-	-

Plate 4.10 Immuno-electrophoresis: anode at left hand side, cathode at right hand side.

Photograph 1. Upper well - neat porcine serum.
Lower well - porcine serum diluted 1 in 2 in phosphate buffered saline, pH 7.2.
Trough - anti pig globulin serum, rabbit 1, diluted 1 in 2 in phosphate buffered saline, pH 7.2.

Photograph 2. Upper well - neat porcine serum.
Lower well - porcine serum diluted 1 in 2 in phosphate buffered saline, pH 7.2.
Trough - anti pig globulin serum, rabbit 6, diluted 1 in 2 in phosphate buffered saline, pH 7.2.



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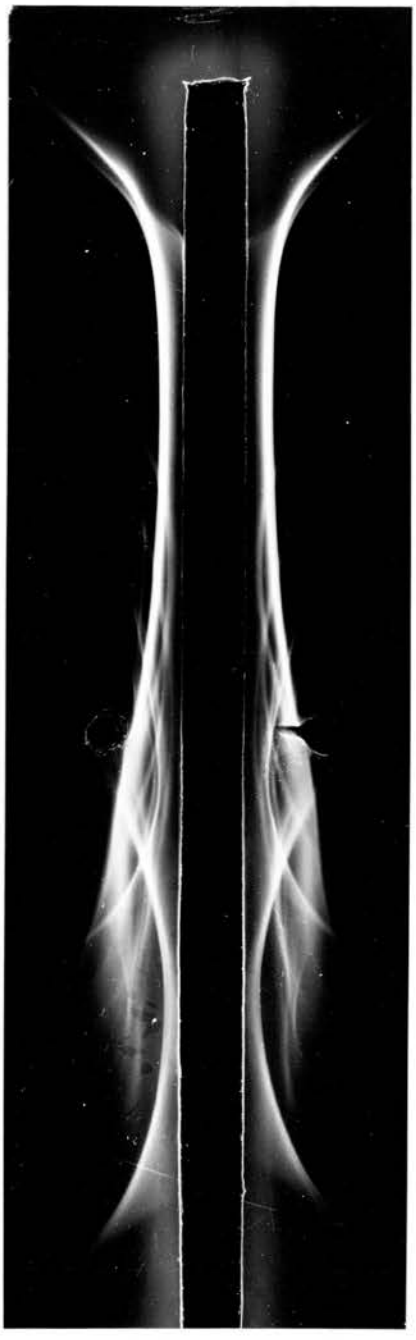

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PLATE 4.10

Plate 4.11 Immuno-electrophoresis: anode at left hand side, cathode at right hand side.

Photograph 1. Upper well - neat porcine serum.

Lower well - porcine serum diluted 1 in 2 in phosphate buffered saline, pH 7.2.

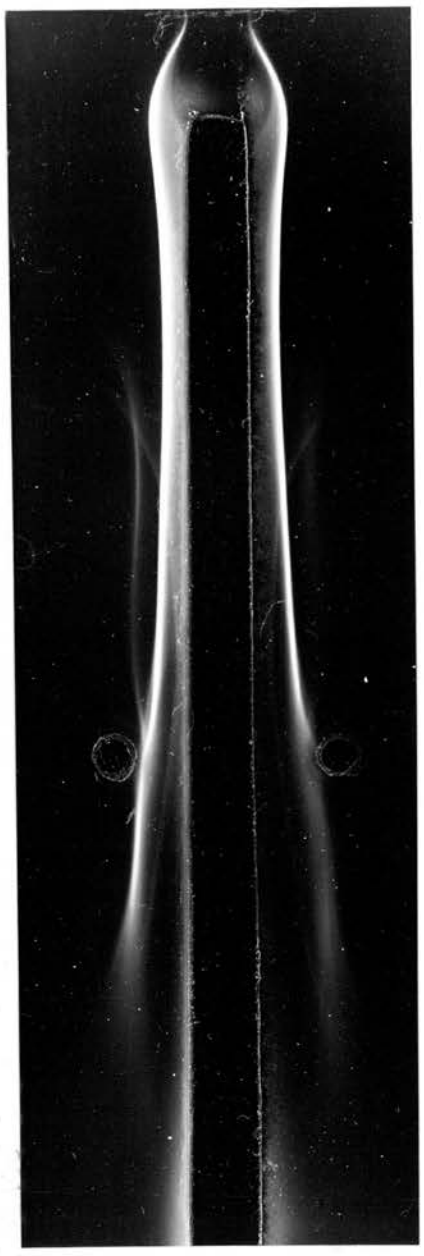
Trough - anti pig globulin serum, rabbit 29, diluted 1 in 2 in phosphate buffered saline, pH 7.2.

Photograph 2. Upper well - neat porcine serum.

Lower well - porcine serum diluted 1 in 2 in phosphate buffered saline, pH 7.2.

Trough - anti pig globulin serum, rabbit 30, diluted 1 in 2 in phosphate buffered saline, pH 7.2.

PLATE 4.11



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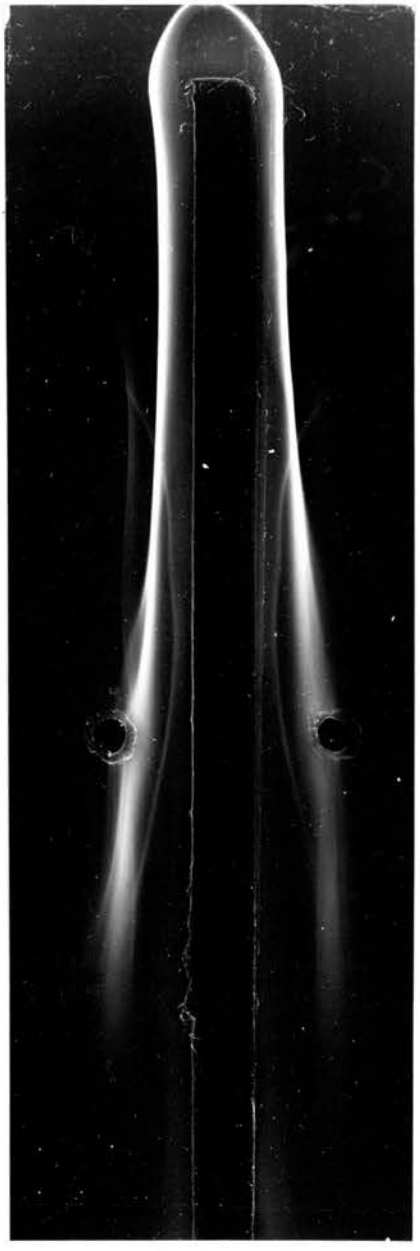

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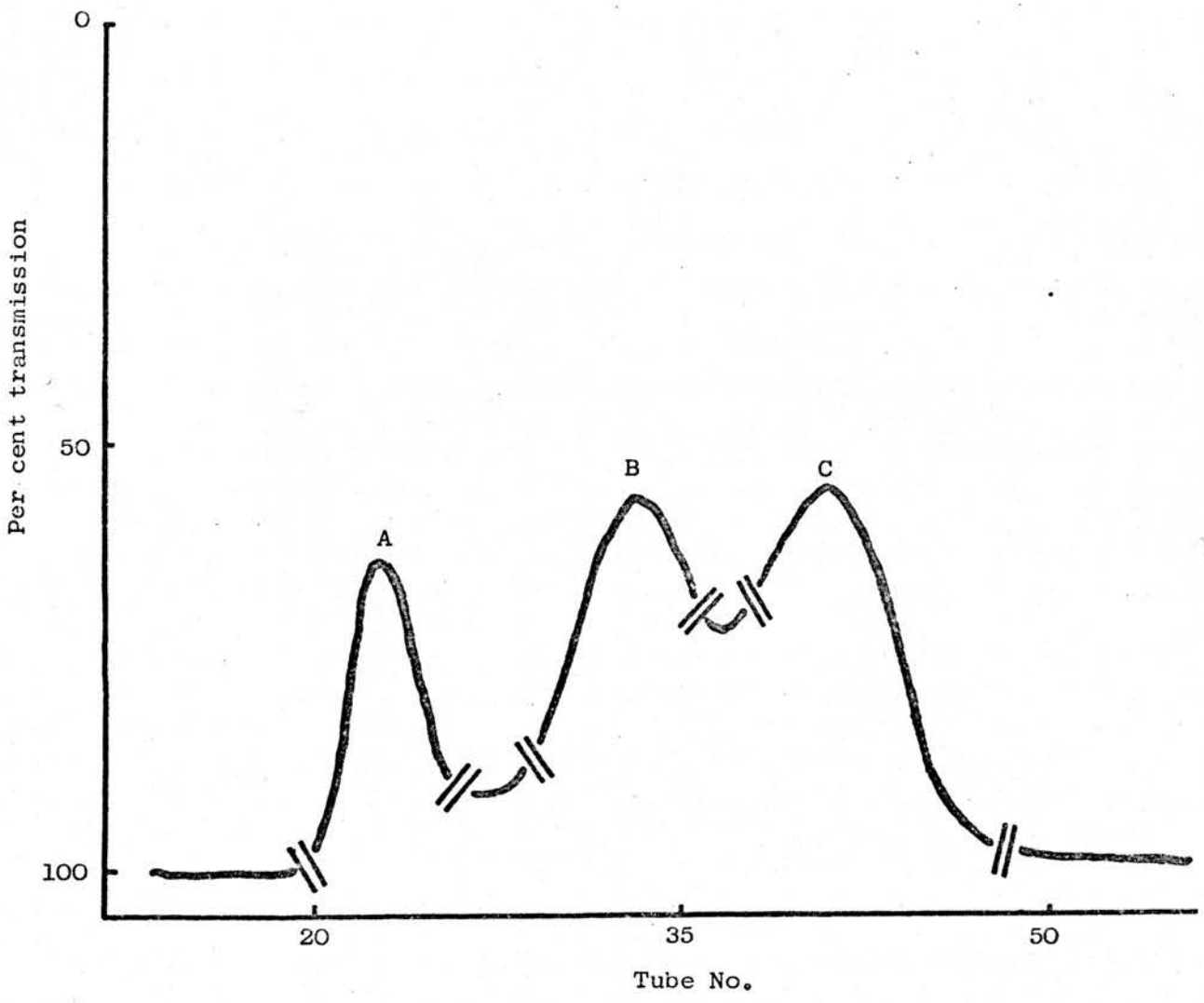


Fig. 4.2 Sephadex G 200 fractionation of immune serum from pig 306. Tubes under the three main peaks were pooled as follows:-

A (19S) - Tubes 19-24

B (7S) - Tubes 28-34

C (4.5S) - Tubes 37-46

5. Naturally occurring thrombocytopenic purpura in piglets.

Studies on natural thrombocytopenic purpura in piglets took two forms. Firstly, the occurrence of the disease was followed in the piglets of four litters and thrombocyte counts in these compared with those of piglets in two normal litters kept under the same husbandry conditions in the same piggery. Secondly, experiments were performed a) by fostering piglets from a sow which had produced a thrombocytopenic litter on to a sow which had produced a normal litter and vice versa; and b) by mating a sow which had already produced a thrombocytopenic litter to her son which had been an affected member of that litter and had recovered. In both these cases all piglets were examined for signs of purpura and blood sampled for thrombocyte counts to be carried out from birth up to about four weeks of age.

(a) Normal litters. All the piglets in two litters, i.e. from gilts 309 and 328, both sired by the same boar, 311, were bled at intervals from birth up to about five weeks of age (Appendix V, Tables 1 and 2). Initially, the thrombocyte counts of all piglets fell in a range from 200 x 10^3 per mm^3 to just over 300 x 10^3 per mm^3 with litter means of 252 $\pm$ 12.5 x 10^3 per mm^3 and 289 $\pm$ 8.7 x 10^3 per mm^3 (Table 5.1). Twenty four hours after the first ingestion of colostrum there was a slight drop in the litter means to 235 $\pm$ 7.8 x 10^3 per mm^3 and 250 $\pm$ 16.0 x 10^3 per mm^3 respectively, after which time they gradually increased reaching peaks of 598 $\pm$ 25.0 x 10^3 per mm^3 and 849 $\pm$ 68.0 x 10^3 per mm^3 at ten days before gradually falling to around 450 x 10^3 per mm^3 at five weeks (Table 5.1).

Therefore the initial and final ranges for both litters were of the same order with similar trends for the means from birth throughout the period of study, the only difference being in the peak levels at ten days of age when the mean for the piglets from 309 was much higher than that for those from 328.

(b) Litters suffering from thrombocytopenic purpura. Two consecutive litters from one sow, 30 and one litter from each of two other sows, 304 and 343, all of which were known to have produced at least one litter affected with thrombocytopenic purpura, were studied from birth up to about four weeks of age. Careful examinations for signs of purpura and thrombocyte counts were carried out at regular intervals during this time.

(i) Sow 30 - first litter. Fourteen normal piglets were born alive but three died from crushing within the first day. By thirty six hours of age, haemorrhages were apparent in varying degrees in all piglets, the visible sites most frequently affected being the ears, face and ventral aspect of the abdomen (Plate 5. 1, 1). One was very severely affected and died. At post mortem examination, large haemorrhages were demonstrated in the subcutaneous tissues along the ventral aspect of the body, throughout most of the organs and in the lumen of the small intestine (Plate 5.1, 2).

Thrombocyte counts carried out on the ten survivors showed them all to be thrombocytopenic (Appendix V, Table 3) with a mean count of $93 \pm 18.5 \times 10^3$ per mm^3 (Table 5. 2). After bleeding, great difficulty was experienced in stopping

Table 5. 1

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of two normal litters with numbers of piglets sampled in brackets.

Time of Sampling	Litter ex Sow 309	Litter ex Sow 328
Birth	289 ± 8.7 (7)	252 ± 12.5 (7)
24 h	250 ± 16.0 (8)	235 ± 7.8 (7)
72 h	343 ± 10.3 (8)	387 ± 8.4 (7)
5-6 d	506 ± 19.4 (8)	459 ± 22.6 (7)
7-8 d	680 ± 22.5 (8)	562 ± 26.0 (7)
10 d	849 ± 68.0 (7)	598 ± 25.0 (7)
12 d	-	521 ± 23.9 (7)
14 d	-	501 ± 33.6 (7)
17-18 d	556 ± 13.5 (8)	493 ± 26.6 (7)
19 d	-	576 ± 39.5 (7)
21 d	-	551 ± 38.3 (7)
24 d	466 ± 16.3 (8)	-
31 d	439 ± 18.5 (7)	-
35 d	476 ± 13.6 (7)	452 ± 18.7 (7)

Table 5.2

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of litters suffering from purpura with numbers of piglets sampled in brackets.

Time of Sampling	Litter ex			
	30 (1)	30 (2)	343	304
Birth	-	-	420 ± 14.7 (8)	334 ± 16.9 (7)
12-24 h	-	125 ± 9.9 (7)	100 ± 9.6 (8)	37 ± 16.3 (6)
36-48 h	93 ± 18.5 (10)	96 ± 5.1 (7)	177 ± 14.4 (7)	-
3-4 d	160 ± 24.6 (10)	276 ± 11.5 (7)	-	273 ± 58.7 (6)
5-7 d	314 ± 28.2 (10)	291 ± 17.5 (7)	435 ± 38.9 (7)	453 ± 43.3 (6)
9 d	189 ± 20.1 (10)	218 ± 25.7 (6)	402 ± 63.3 (7)	-
11 d	218 ± 15.0 (10)	141 ± 21.7 (6)	-	-
12-13 d	275 ± 21.4 (8)	144 ± 26.3 (6)	212 ± 24.8 (6)	-
14 d	-	135 ± 24.1 (5)	106 ± 21.9 (6)	-
16 d	301 ± 16.4 (8)	156 ± 28.8 (5)	161 ± 23.7 (6)	-
18-19 d	346 ± 19.8 (8)	212 ± 36.9 (5)	236 ± 41.3 (6)	-
22-23 d	430 ± 7.8 (8)	241 ± 50.4 (5)	408 ± 52.2 (6)	-
28 d	396 ± 21.2 (8)	332 ± 28.2 (5)	-	-

the flow of blood due to the impaired haemostatic mechanism and mild cautery had to be performed on the puncture areas to prevent the piglets from bleeding to death.

No more deaths occurred at this time and the visible haemorrhages gradually resolved as the thrombocyte levels recovered. A further thrombocytopenia occurred at nine days (Table 5.2) without symptoms of purpura after which time there was again a recovery in the thrombocyte counts until they stabilised at around 400×10^3 per mm^3 at three weeks of age.

The whole litter suffered from a severe enteritis at ten to fourteen days old which resulted in two further deaths not associated with thrombocytopenia. Hence the thrombocyte observations were completed on the eight remaining piglets which responded to antibiotic therapy for this condition.

(ii) Sow 30 - second litter. Eight piglets were born alive, one being crushed by the mother within the first few hours of life. When sampled twelve hours after birth all of the remainder were thrombocytopenic (Appendix V, Table 4) with a litter mean count of $125 \pm 9.9 \times 10^3$ per mm^3 (Table 5.2). All were affected with small subcutaneous haemorrhages, and the thrombocytopenia reached its lowest point thirty six hours after birth, after which time there was a gradual increase to $291 \pm 17.5 \times 10^3$ per mm^3 at one week. No deaths occurred at the time of the primary thrombocytopenia but one piglet died from intestinal impaction, not apparently associated with this, at seven days of age.

As with the first litter, so with this one a secondary thrombocytopenia occurred at eleven days and lasted up to

sixteen to eighteen days old before the thrombocyte counts gradually recovered again. Several piglets showed haemorrhages especially along the ventral aspect of the body (Plate 5. 2, 1) and one died at twelve days showing a very low thrombocyte count which was carried out just before death (Appendix V, Table 4). At post mortem examination large ecchymotic haemorrhages were found throughout all the organs, especially the heart, stomach and intestines as well as in the subcutaneous tissues (Plate 5.2, 2).

(iii) Sow 343. Eight piglets were born with normal thrombocyte counts (mean 420 ± 14.7 x 10^3 per mm^3) and all became thrombocytopenic twenty four hours after ingesting colostrum (Appendix V, Table 5) with a litter mean thrombocyte count of 100 ± 9.6 x 10^3 per mm^3 (Table 5.2). No symptoms of purpura were apparent at this time and the counts gradually increased up to five to nine days of age. Only one death occurred at this time and that was due to crushing at one day old.

A secondary thrombocytopenia ensued at from ten to fourteen days, the time of onset being variable. Several piglets showed signs of purpura and one of the most severely affected died. Post mortem examination demonstrated lesions similar to that of the piglet in the second litter from 30 (Plate 5.2, 2). The remaining six piglets gradually recovered, the mean thrombocyte count being 408 ± 52.2 x 10^3 per mm^3 at three weeks.

(iv) Sow 304. All seven piglets born alive had normal thrombocyte counts (litter mean 334 ± 16.9 x 10^3 per mm^3).

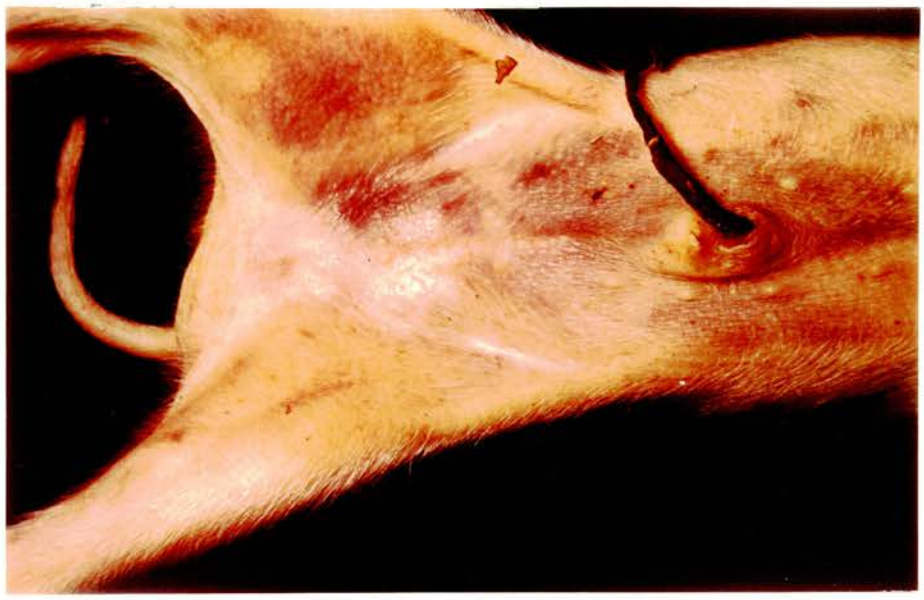
One was very weak and succumbed within the first twenty four hours. All the others became thrombocytopenic after ingesting colostrum (Appendix V, Table 6) with a litter mean thrombocyte count of $37 \pm 16.3 \times 10^3$ per mm^3 (Table 5.2) at twenty four hours. Small haemorrhages were apparent along the ventral aspect of the abdomen and on the ears but these gradually disappeared as the thrombocyte counts increased, as in the other litters.

Plate 5.1 Piglet from sow 30's first litter, thirty six hours old.

Photograph 1. Haemorrhages along the ventral aspect of the abdomen.

Photograph 2. Post mortem examination - haemorrhages in the subcutaneous tissues along the ventral aspect of the body; extensive haemorrhage into the small intestine.

PLATE 5.1



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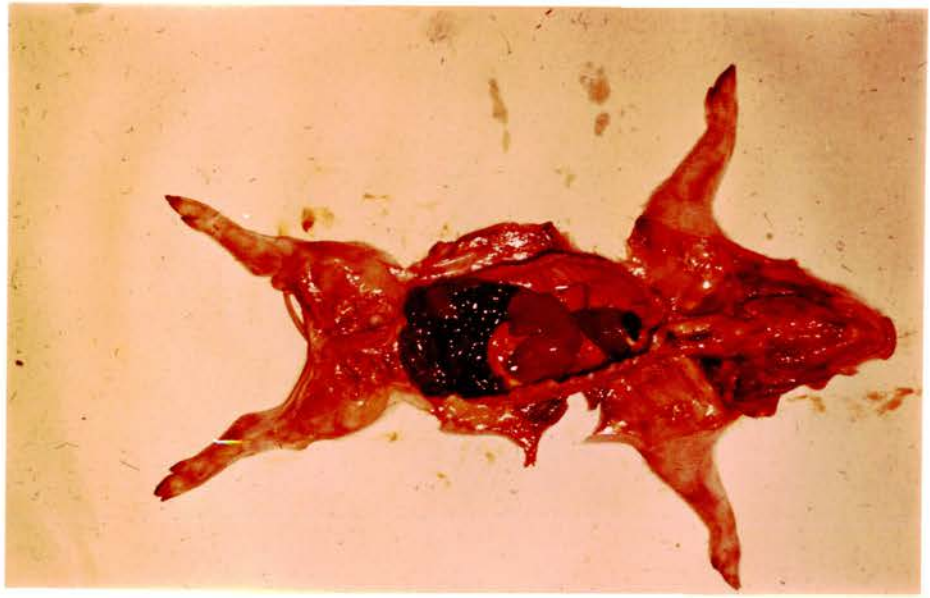

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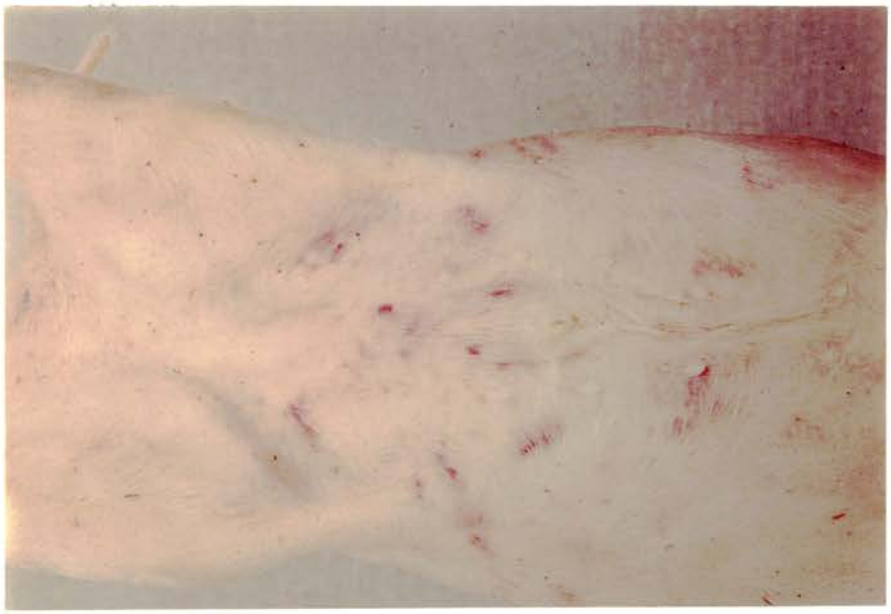
Plate 5.2 Piglet from sow 30's second litter, twelve days old.

Photograph 1. Haemorrhages along the ventral aspect of the abdomen.

Photograph 2. Post mortem examination - haemorrhages in the heart, stomach, intestines and subcutaneous tissues.

PLATE 5.2



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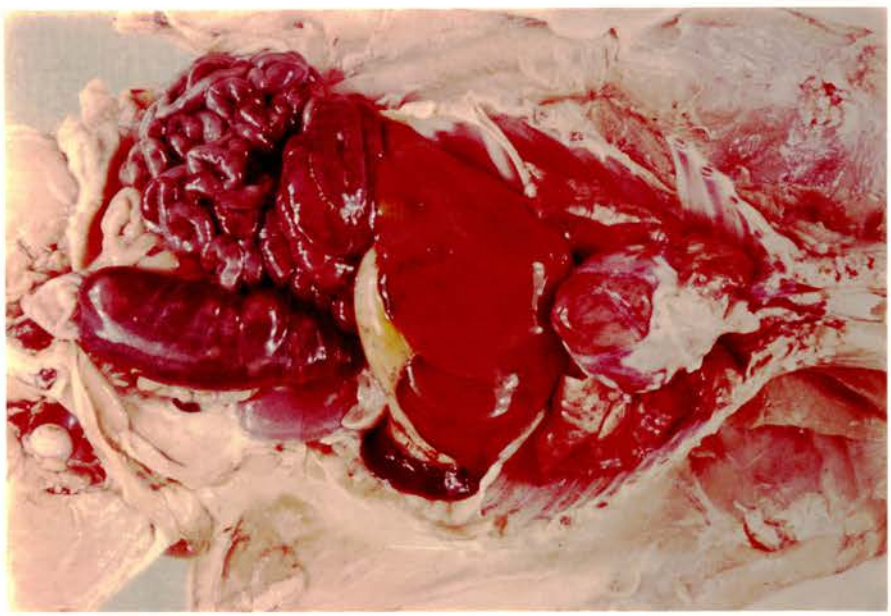

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(c) Comparison of thrombocyte counts of affected and normal litters. All affected litters showed a marked thrombocytopenia after ingesting colostrum, much greater than the slight drop in thrombocyte counts of the normal litters (Fig. 5.1). The latter was presumably due to a relative increase in the intravascular fluid volume following the ingestion and absorption into the circulation of globulins from the colostrum of the sow by the piglets, with no increase in the absolute number of circulating thrombocytes. After the initial drop, all affected litters showed an increase up to around seven days of age, after which a further thrombocytopenia followed in three of the litters, the fourth, 304, not being followed after this time. In all three litters mean thrombocyte counts were then apparently depressed with respect to the normal litters, up to three weeks of age in two litters and up to four weeks in the third.

Analysis of variance was used to assess the significance of the difference in mean counts of normal and affected litters (Table 5.3). Degrees of freedom and sums of squares were partitioned as follows:

	Degrees of Freedom
Normal versus affected	1
Between 3 normal litters	2
Between 3 or 4 affected litters	2 or 3
Total between	4 or 5
Within litters	N - 6 or 7
Total	N - 1

Where N was the total number of piglets, F was the ratio between normal v affected (1 DF) and total between (4 or 5 DF).

As the sows all farrowed at different times, all piglets were not bled at exactly the same age. Further, as two of the affected litters were born unexpectedly during the night, no precolostral samples were available from them, and all bleeds from piglets which died were included up to the time of death. Hence the numbers of litters and piglets in each group are not constant throughout. A litter of four normal piglets from sow 1083 (see Results 5.d) were included to increase the number of normal litters.

There was no significant difference between precolostral samples although the mean count of piglets in the affected litters was higher than that of the normal ones. At twelve to twenty four hours after the ingestion of colostrum there was a significant difference between the two ($0.05 > P > 0.01$) but this was no longer the case at two further bleeds up to eight to ten days when the secondary thrombocytopenia occurred in the affected piglets and the difference again became significant ($0.05 > P > 0.01$). This level of difference persisted up to about three weeks of age when the two means again became not significantly different.

Table 5.3

Significance of difference between mean thrombocyte counts x 10^3 per mm^3 of normal and affected piglets by analysis of variance.

Age at Sampling	Normal litters			Affected litters			F	$N_1=1$ / $N_2=$	Significance
	No.	Piglets	Mean	No.	Piglets	Mean			
Birth	3	19	296	2	15	380	2.48	3	NS
12-24 h	3	19	269	3	21	90	15.80	4	*
48-72 h	2	15	364	3	24	171	7.135	3	NS
5-7 d	3	18	505	4	30	365	4.90	5	NS
8-10 d	3	19	685	3	23	261	12.69	4	*
11-13 d	2	11	645	3	20	217	10.94	3	*
14-16 d	2	10	526	3	19	213	16.74	3	*
17-19 d	2	15	565	3	19	276	30.06	3	*
20-24 d	2	15	505	3	19	373	2.97	3	NS
24 d	2	15	445	2	13	371	5.70	2	NS

* $0.05 > P > 0.01$

NS not significant

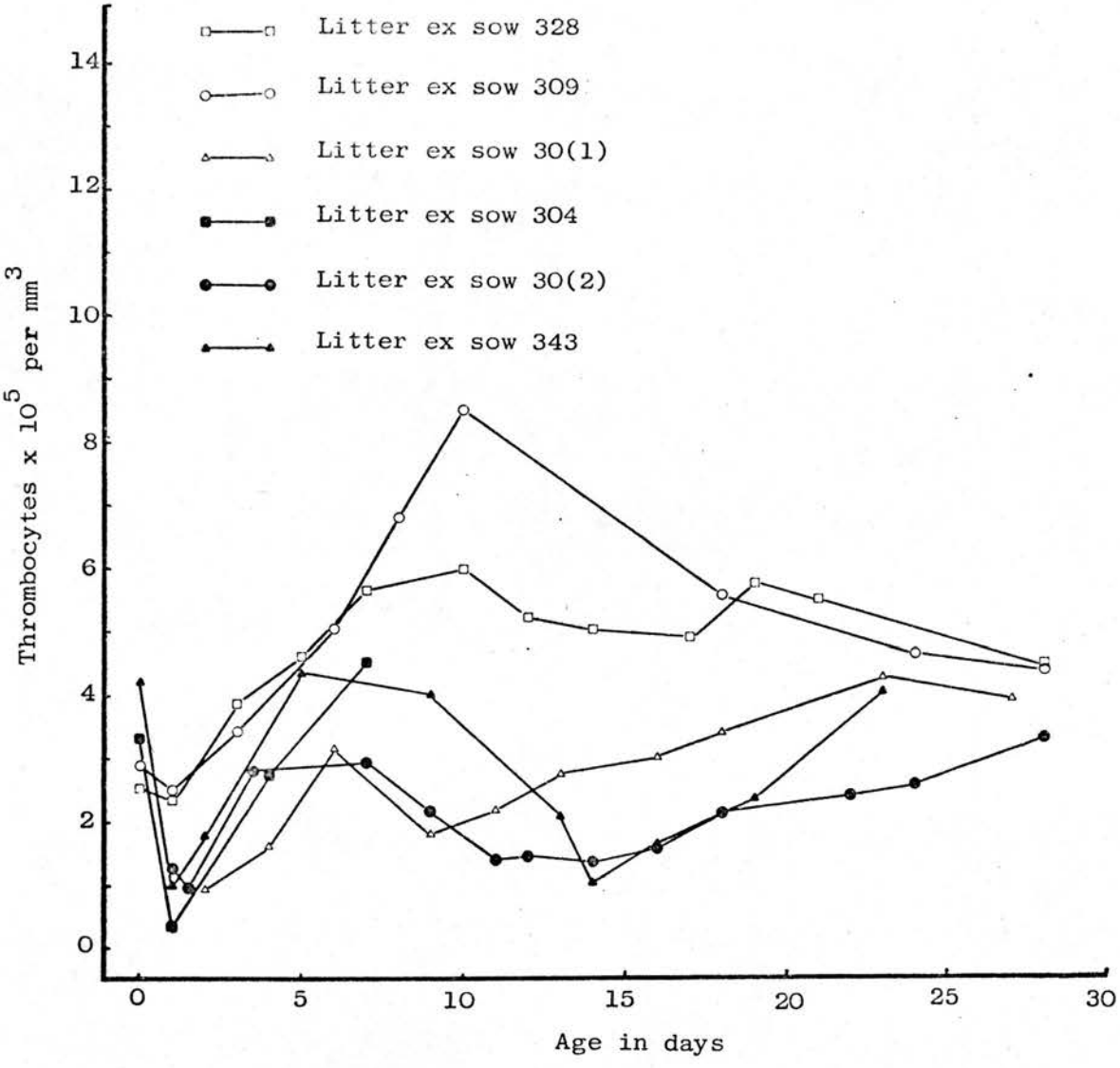


Fig. 5.1 Mean thrombocyte counts of piglets in two normal litters (ex sows 309 and 328) and four litters affected with thrombocytopenic purpura (ex sows 30 (1) and (2), 304 and 343) from birth to thirty days of age.

(d) Cross-suckling experiment. Two sows, viz. 305 which had produced a thrombocytopenic litter and 1083 which had already produced a normal litter, were mated with different boars at the same time. Unfortunately, each sow had only six piglets born alive when they farrowed within twelve hours of each other. Two piglets from each were transferred to the other before getting colostrum from their respective dams and thrombocyte counts of all piglets were followed from birth onwards (Appendix V, Table 7).

Piglets from sow 305 suckled by her showed mild signs of purpura at twenty four hours after ingesting her colostrum, with concurrent thrombocytopenia (Table 5.4). This was followed by a gradual recovery both in the haemorrhages and in the thrombocyte counts up to eight days of age when a further thrombocytopenia, this time without marked purpura, was apparent just like the piglets in the other naturally affected litters (Results, 5.b). This thrombocytopenia lasted up to nineteen days of age when the observations ceased. On the other hand, the two piglets fostered by sow 1083 remained normal throughout the period of study, both in clinical signs and thrombocyte counts.

Similarly, all piglets from 1083, irrespective of whether they were suckled by their dam or by 305, neither showed signs of purpura nor were thrombocytopenic over the period.

Therefore of the four groups of piglets only one, that born of and suckled by 305, showed purpura with typical primary and secondary thrombocytopenia (Fig. 5.2).

On statistical analysis by analysis of variance (Table 5.4) there was no difference between the thrombocyte counts of all four groups at birth. From twenty four hours to four days later, the thrombocyte counts of the piglets out of 305 and suckled by her (Group A) were very significantly lower than the other three groups ($P < 0.001$). At eight days there was no significant difference in the mean counts but a significant difference was again apparent at eleven days ($P < 0.001$) which was still present at fifteen days, though at a lower level ($0.05 > P > 0.01$), when the observations ceased.

The thrombocyte counts of the piglets from 305 which were fostered by 1083 (Group B) followed a similar pattern to both groups (C & D) from 1083 (Fig. 5.2), though at a lower level. The pattern was similar to the differences seen between the counts of the two normal litters (Results 5.a) and is no greater than would be expected between two normal litters of piglets, being only statistically significant at the twenty four hour stage ($0.05 > P > 0.01$).

This experiment supported the hypothesis that the condition was due to anti thrombocyte antibodies absorbed from the dam's colostrum and that these antibodies were specific for certain thrombocyte antigens, in this case those carried by the thrombocytes of the offspring of 305. Piglets from 1083 which were of a different breed were unaffected when ingesting the same colostrum and their thrombocyte antigens were therefore probably of a different type.

Table 5.4

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of piglets in cross-suckling experiment involving sows 305 and 1083, with numbers sampled in brackets.

Age	Sow 305		Sow 1083		Statistical Analysis
	Own piglets (A)	Nursed by 1083 (B)	Nursed by 305 (C)	Own piglets (D)	
Birth	348 ± 20.0 (4)	385 ± 6.0 (2)	368 ± 37.5 (2)	384 ± 13.1 (4)	NS
24 h	86 ± 4.8 (4)	241 ± 0.0 (2)	361 ± 21.2 (2)	368 ± 8.5 (4)	A v B, C & D *** Between B,C & D *
4 d	225 ± 26.9 (3)	301 ± 26.0 (2)	386 ± 0.0 (2)	367 ± 13.0 (3)	A v B, C & D *** Between B,C & D NS
8 d	504 ± 47.9 (2)	465 ± 36.0 (2)	646 ± 36.0 (2)	512 ± 88.7 (4)	NS
11 d	314 ± 22.0 (2)	550 ± 26.5 (2)	837 ± 2.0 (2)	863 ± 87.0 (4)	A v B, C & D *** Between B,C & D NS
13 d	115 ± 28.5 (2)	480 ± 10.0 (2)	-	-	-
15 d	127 ± 80.1 (2)	450 ± 5.0 (2)	520 ± 83.0 (2)	584 ±101.7 (3)	A v B, C & D * Between B,C & D NS

*** $p < 0.001$ * $0.05 > p > 0.01$ NS not significant

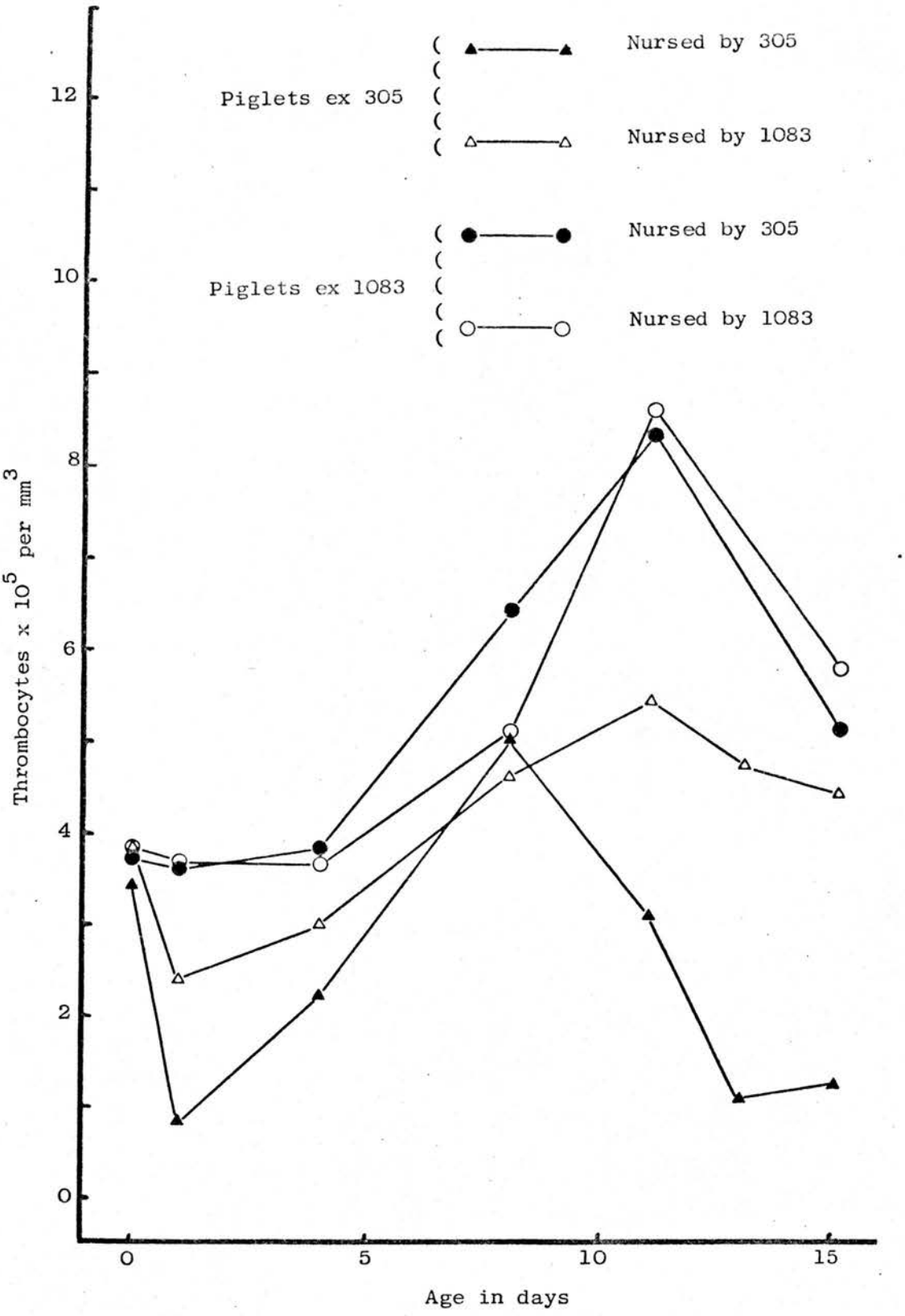


Fig. 5.2 Mean thrombocyte counts of piglets in litters from sows 305 and 1083 in cross suckling experiment.

(e) Back-cross mating. If genetically determined types of thrombocytes are involved in this condition, it would be expected that, in the clinical cases, the dam is negative for the specific factor or factors involved while affected piglets are heterozygous for it. To test this theory, sow 305 was mated to a son, 312, which had been an affected member of a previous litter, and the thrombocyte counts of all piglets in the resulting litter followed (Appendix V, Table 8).

Nine normal piglets were born with a mean thrombocyte count of $241 \pm 9.8 \times 10^3$ per mm^3. Twenty four hours after ingesting colostrum five were found to have patches of purpura all over while four were normal. Thrombocyte counts showed the former to be thrombocytopenic while the latter remained normal (Table 5.5). The signs of purpura gradually regressed and no marked secondary thrombocytopenia was apparent in the affected piglets. The means of the affected piglets did however, remain lower than the unaffected ones up to nineteen days of age when the observations ceased (Fig.5.3).

The difference between the mean thrombocyte counts of non-affected and affected piglets was statistically analysed by "Student's" t test (Table 5.5). No significant difference was present between the two groups before they received colostrum. From twenty four hours to four days later there was a highly significant difference ($p < 0.001$) but no significant difference was present at eight days. From fourteen to nineteen days the thrombocyte levels of the piglets which had been affected were significantly lower than

those which had not been affected ($0.05 > p > 0.01$) and from this it appeared that a thrombocytopenia was present in the former relative to the latter. Hence the picture in the affected piglets was similar to those affected piglets in litters already studied (Results 5.b).

In this mating of a son back to his mother, the litter was divided equally both according to clinical signs and thrombocyte counts, there being complete correlation between these two parameters. This evidence strongly supports the hypothesis that the son, 312, was heterozygous for the factor which was involved in the thrombocytopenia while the dam, 305, lacked it.

Conclusions. A haemorrhagic condition of young piglets manifest by purpura and thrombocytopenia has been studied. Piglets became affected after ingesting colostrum while litter mates fostered on to another sow were not affected. Thrombocytopenia with or without concurrent purpura was present within the first few days of life and again at from eight to twenty one days of age. Death occurred at either or both times depending on the severity of the condition. Multiple haemorrhages were apparent throughout the subcutaneous tissues and all the organs. There was a significant difference between the mean thrombocyte counts of normal and affected piglets kept under the same conditions of husbandry.

The evidence presented supports the hypothesis that the condition is due to iso-immunisation of the dam by foetal thrombocyte antigens either at parturition or during pregnancy

as in the case of the red cell iso-antibodies. The iso-antibodies so produced, ingested by the piglets in the colostrum, are absorbed and produce thrombocytopenia with consequent purpura, It is highly probable that the piglets with thrombocytes of an antigenic type similar to their sire are affected while those whose thrombocytes are similar to the dam are not affected.

Table 5.5

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of piglets, unaffected and affected with purpura, ex sow 305 sired by her son, 312, with numbers sampled in brackets.

Age	Non-Affected	Affected	Diff.	SE of diff.	DF	t	Sig.
Birth	247 ± 14.7 (4)	237 ± 14.3 (5)	10	± 71.875	7	0.139	NS
24 h	229 ± 15.7 (4)	41 ± 9.6 (5)	188	± 17.57	7	10.701	***
4 d	380 ± 19.1 (4)	168 ± 15.3 (5)	212	± 21.31	7	9.948	***
8 d	585 ± 15.7 (4)	531 ± 38.5 (5)	54	± 40.39	7	1.337	NS
14 d	590 ± 54.8 (4)	397 ± 65.1 (5)	193	± 77.66	7	2.485	*
19 d	634 ± 15.5 (4)	465 ± 45.5 (4)	169	± 48.08	6	3.514	*

NS - not significant *** - $p < 0.001$ * - $0.05 > p > 0.01$

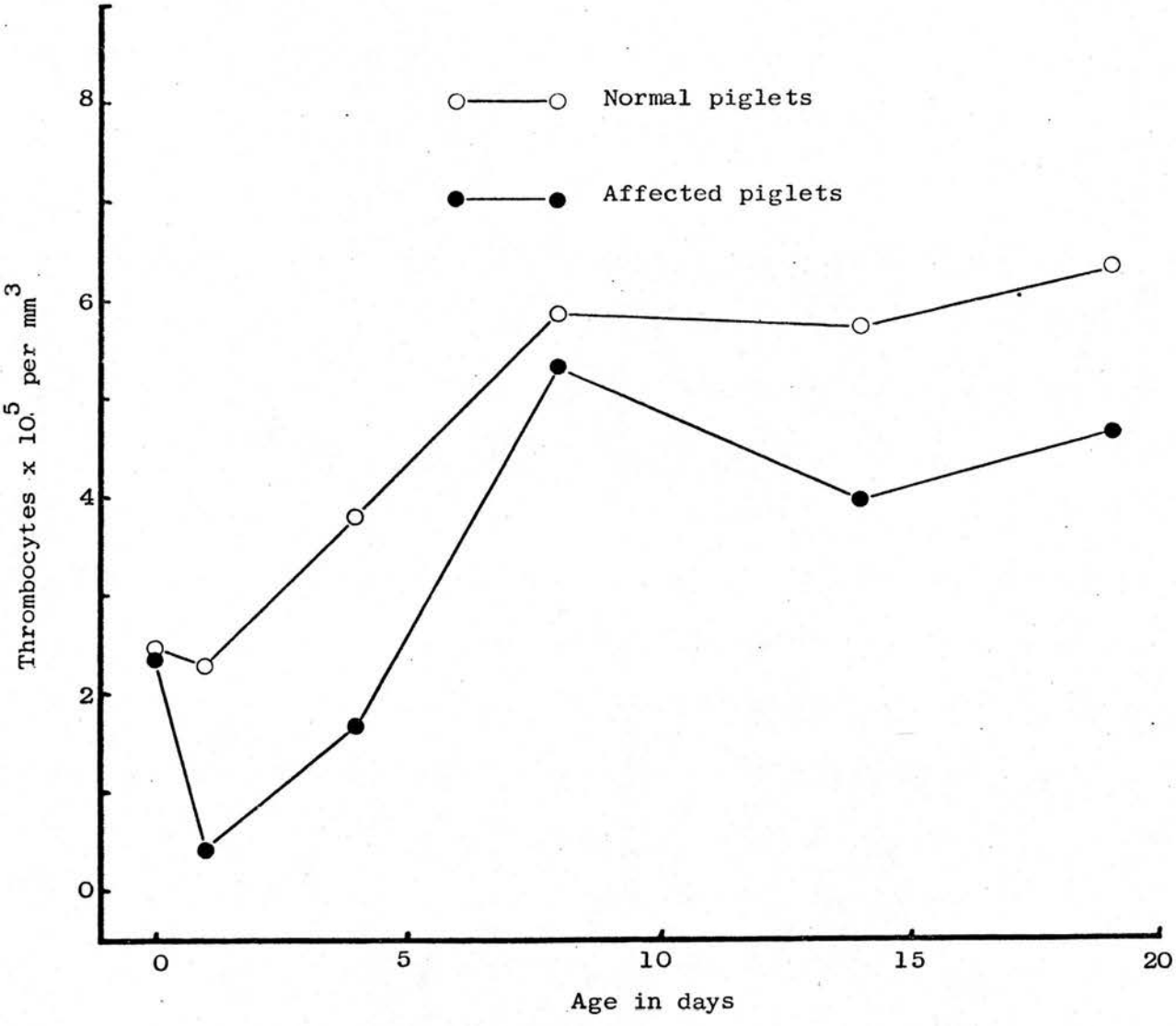


Fig. 5.3 Mean thrombocyte counts of normal piglets and those affected with thrombocytopenic purpura in litter from sow 305 by her son 312.

6. Experimentally induced thrombocytopenic purpura in piglets.

Two gilts, 306 and 307, were immunised with platelets from a litter brother, 311 (Materials and Methods 6, b). Antibodies to the platelets were detected in both gilts by means of the antiglobulin consumption test (Results 4, v), before mating with 311.

(a) Experimental reproduction of thrombocytopenic purpura.

(i) Experimental Litter No.1 (first litter ex 307). Gilt 307 was given a booster doze of platelet suspension ten days before the expected parturition date in order that the antibody level might be at a maximum at that time. In fact she farrowed five days earlier than expected.

Eleven piglets were born, one of which was still-born. Thrombocyte counts were all within the normal range (Appendix VI, Table 1; Table 6.1). Within twelve hours, five were showing severe signs of purpura, two mildly affected and three were clinically normal. In affected piglets, large subcutaneous haemorrhages were evident especially on the forehead, ears and along the ventral aspect of the body (Plate 6.1). Thrombocyte counts were very low in all affected animals, there being no difference between those which were severely affected and those which were mildly affected. Of the clinically normal ones, two had thrombocyte counts at the lower end of the normal range while one was thrombocytopenic. By the second day the latter was also showing clinical signs of purpura. After bleeding the

puncture sites in all thrombocytopenic animals had to be cauterised to prevent massive haemorrhage due to the defective haemostatic mechanism.

Within three days of birth, six affected piglets died. Post mortem examinations revealed subcutaneous haemorrhages all over the body but especially over the occipital region, along the chest wall and the ventral aspect of the body. Haemorrhages were present in most organs and in one animal, the main lesion was massive haemorrhage into the small intestine (Plate 6.2).

Thrombocyte counts in the two affected survivors gradually increased up to eight days of age when a further thrombocytopenia occurred in both (Table 6.1). No clinical signs of purpura were apparent at this time and counts returned to levels similar to the clinically non-affected ones within three to four days.

The differences between the mean thrombocyte counts of affected and non-affected piglets were statistically analysed by "Student's" t test (Table 6.1). Precolostral samples showed no difference between the two groups. Twelve to thirty six hours later there was a very significant difference between them ($p < 0.001$) and at six days of age, the affected piglets still had thrombocyte counts significantly lower than the non-affected ones ($0.01 > p > 0.001$). The thrombocyte counts of the affected piglets fell again at eight days but this was not reflected in the statistical analysis due to the small number of pigs involved and the large variance in the counts of the non-affected ones. At ten days the counts of

affected were still significantly lower than that of the non-affected ($0.05 > p > 0.01$) but after this no significant differences could be demonstrated between the two groups.

When compared with the mean thrombocyte counts of a normal litter (Fig. 6.1) those of the non-affected piglets followed a similar course apart from the first thirty six hours after ingesting colostrum when they showed a more marked fall indicating that their thrombocytes were perhaps being affected to a slight degree. They did not, however, reach as low levels as their non-affected litter-mates.

The thrombocyte counts of affected piglets in the experimental litter followed closely those of a naturally affected litter (Fig. 6.1) even to the extent of showing a secondary thrombocytopenia. The former did, however, reach higher levels sooner than the latter.

The antiglobulin consumption test was not carried out until the piglets were eight weeks of age because of the quantity of blood required to yield enough platelets for the test. By this time one of the normal piglets had died due to reasons unconnected with the experiment. Thrombocytes from the three remaining piglets were tested against serum taken from the dam at farrowing (Appendix VI, Table 2). Those from affected piglets gave a positive result while the non-affected one was negative. Hence there was complete correlation between clinical signs of thrombocytopenia and purpura and the serological reaction of the three piglets' thrombocytes with the dam's serum (Table 6.2).

(ii) Experimental litter No.2 (first litter ex 306).

In this case no further injection of platelets was given after the first month of pregnancy. Eight piglets were born, one of which was stillborn. All were clinically normal at birth and precolostral thrombocyte counts were within the normal range (Appendix VI, Table 3; Table 6.3). Twelve hours after first ingesting colostrum, two piglets were still clinically normal, but the remaining five were all showing varying degrees of purpura in the same areas as those of the previous litter (Plate 6.1). Thrombocyte counts in non-affected piglets were still within the normal range and remained so throughout the period of study. On the other hand, the affected piglets were very thrombocytopenic and bleeding points had to be cauterised to stem the flow of blood.

One of the affected piglets died at three days of age, when post mortem examination demonstrated multiple haemorrhages throughout the organs and subcutaneous tissues like those of the previous litter (Plate 6.2). The remaining four affected piglets gradually recovered within seven days with thrombocyte counts similar to non-affected litter-mates, no secondary thrombocytopenia being present.

In this case statistical analysis showed a significant difference between the mean thrombocytes of affected and non-affected piglets at twelve hours ($p < 0.001$). From two days onwards there was no difference just as there was no difference between the precolostral samples of the two groups.

The thrombocyte counts of the non-affected piglets closely followed those of a normal litter (Fig. 6.2). The

Table 6.1

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in first litter ex 307 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Diff.	S.E. of diff.	DF	t	Sig.
Birth	287 ± 38.00 (2)	316.6 ± 25.50 (5)	29.6	± 47.20	5	0.627	NS
12 h	157 ± 10.00 (2)	31.1 ± 7.60 (6)	125.9	± 14.66	6	8.586	***
36 h	127.5 ± 4.53 (2)	47.0 ± 6.77 (5)	80.5	± 11.57	5	6.956	***
6 d	740.5 ± 38.50 (2)	272.5 ± 14.51 (2)	468.0	± 41.15	2	11.373	**
8 d	889 ± 191.0 (2)	90.5 ± 1.58 (2)	798.5	± 191.1	2	4.178	NS
10 d	623.5 ± 1.41 (2)	142.0 ± 55.68 (2)	481.5	± 55.70	2	8.645	*
13 d	542.5 ± 67.50 (2)	453.5 ± 43.50 (2)	89.0	± 80.3	2	1.108	NS
16 d	541.0 ± 69.00 (2)	653.5 ± 3.46 (2)	-112.5	± 69.01	2	1.630	NS
20 d	585 - (1)	540 ± 11.00 (2)	45.0	-	-	-	-
24 d	557 - (1)	508.5 ± 77.50 (2)	48.5	-	-	-	-
27 d	650 - (1)	476 ± 25.00 (2)	174.0	-	-	-	-
35 d	380 - (1)	492.5 ± 79.50 (2)	-112.5	-	-	-	-

*** - $p < 0.001$ ** - $0.01 > p > 0.001$ * - $0.05 > p > 0.01$ NS - not significant

Table 6.2

Clinical signs and serological reactions of platelets of piglets in first litter ex 307 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
402	+	+	N.D.
403	++	+	N.D.
404	+	+	+
405	-	-	N.D.
406	++	+	N.D.
407	++	+	+
408	-	-	-
409	++	+	N.D.
410	+	+	N.D.
411	+	+	N.D.

N.D. - Not done

A.G.C.T. - Antiglobulin consumption test.

\+ - positive

\- - negative

Table 6.3

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in first litter ex 306 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Diff.	S.E. of diff.	DF	t	Sig.
Birth	262.5 ± 22.49 (2)	310 ± 11.01 (5)	-47.5	± 21.94	5	2.165	NS
12 h	202.5 ± 39.50 (2)	24.8 ± 4.56 (5)	177.7	± 22.18	5	8.01	***
2 d	297 ± 6.00 (2)	174.75 ± 44.35 (4)	122.25	± 66.64	4	1.834	NS
4 d	443 ± 20.00 (2)	323.5 ± 73.18 (4)	119.5	±110.54	4	1.081	NS
6 d	547.5 ± 12.49 (2)	433.25 ± 63.42 (4)	114.25	± 95.44	4	1.197	NS
14 d	558.5 ± 25.51 (2)	512.25 ± 19.51 (4)	46.25	± 33.17	4	1.394	NS
20 d	645 ± 39.00 (2)	602 ± 21.63 (4)	43.0	± 40.29	4	1.067	NS
23 d	534 ± 15.00 (2)	518.67 ± 3.92 (3)	15.33	± 12.27	3	1.249	NS
27 d	456.5 ± 22.49 (2)	467.33 ± 28.31 (3)	-10.83	± 40.20	3	0.269	NS
30 d	530 ± 11.00 (2)	517.33 ± 9.48 (3)	12.67	± 14.79	3	0.856	NS
34 d	456 ± 31.00 (2)	533.33 ± 19.39 (3)	-76.33	± 34.06	3	2.26	NS

*** - $p < 0.001$ NS - not significant

affected piglets on the other hand were similar to a naturally affected litter up to six days of age but after this they followed their non-affected litter-mates and did not show the secondary thrombocytopenia (Fig. 6.2).

The antiglobulin consumption test was again carried out when the piglets were eight weeks of age. By this time one of the affected piglets had died due to reasons unconnected with the experiment. Thrombocytes from the five remaining piglets were tested against serum taken from the dam at farrowing (Appendix VI, Table 4). There was a complete correlation between clinical signs of purpura and thrombocytopenia with a positive serological reaction between the piglets' platelets and their dam's serum (Table 6.4).

Table 6.4

Clinical signs and serological reactions of platelets of piglets in first litter ex 306 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
454	+	+	+
455	-	-	-
456	+	+	+
457	-	-	-
458	++	+	N.D.
459	++	+	N.D.
460	++	+	+

N.D. - Not done

A.G.C.T. - Antiglobulin consumption test.

\+ - positive

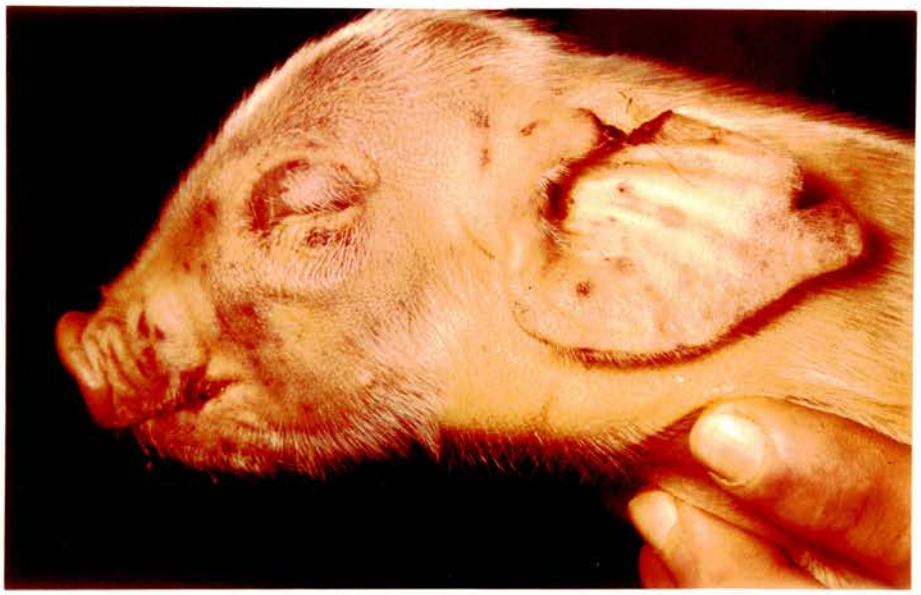
\- - negative

Plate 6.1 Piglet in first litter from sow 307, twelve hours after first ingesting colostrum.

Photograph 1. Small haemorrhages over the head and ears; swelling of the upper eyelid.

Photograph 2. Haemorrhages of varying sizes along the ventral aspect of the abdomen.

PLATE 6.1



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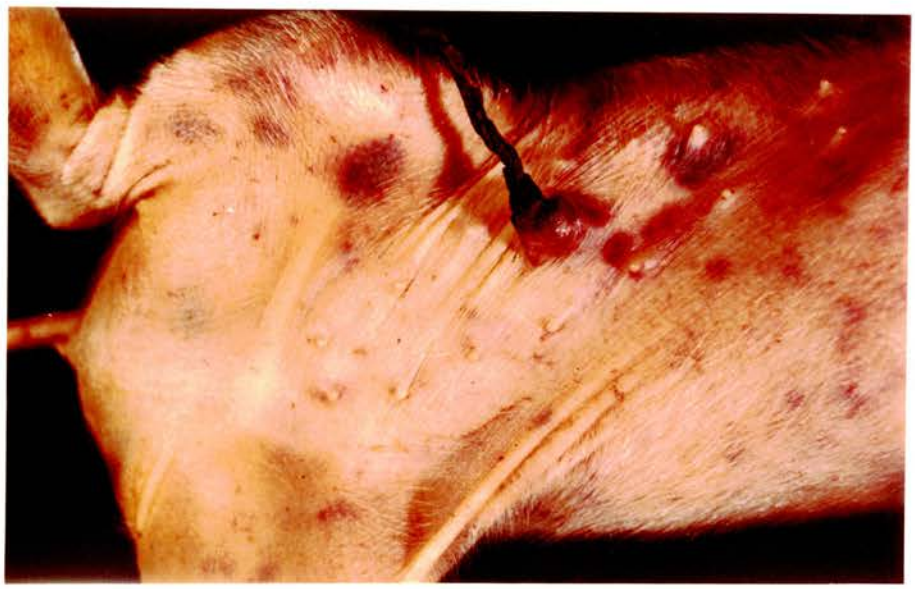


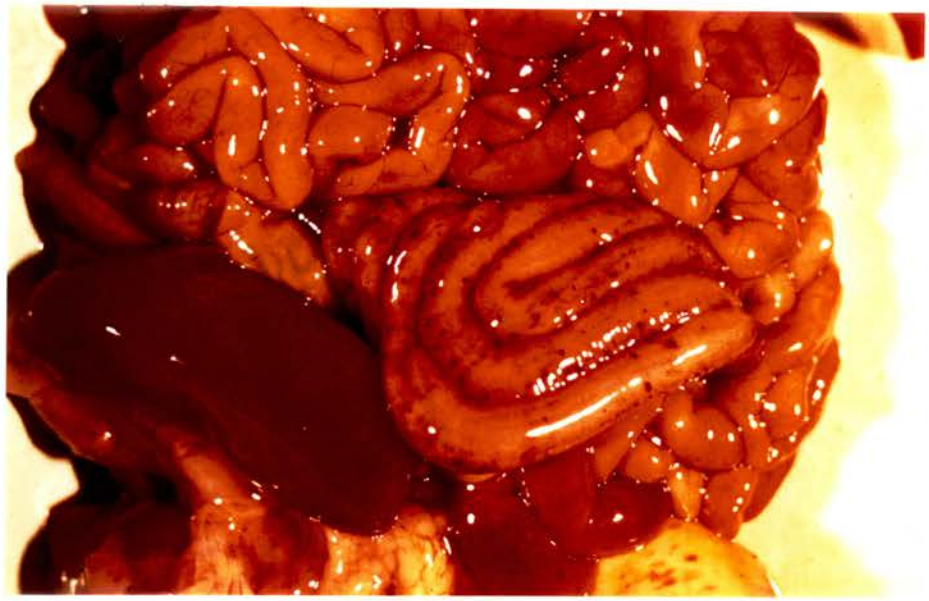
2

Plate 6.2 Post mortem examination of piglets in first litter from sow 307, two days old.

Photograph 1. Small discrete haemorrhages in the large intestine and right kidney.

Photograph 2. Massive haemorrhage into the lumen of the small intestine.

PLATE 6.2



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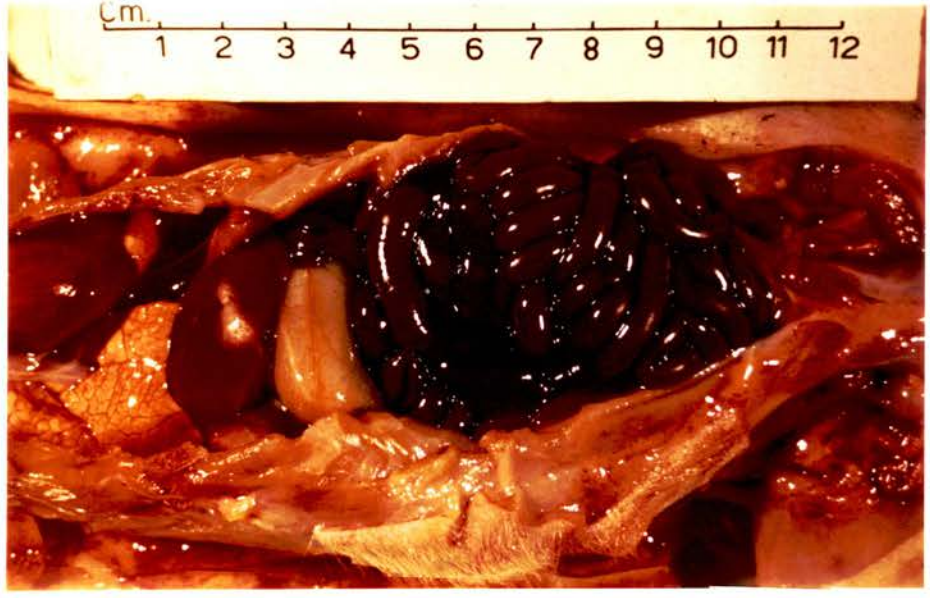

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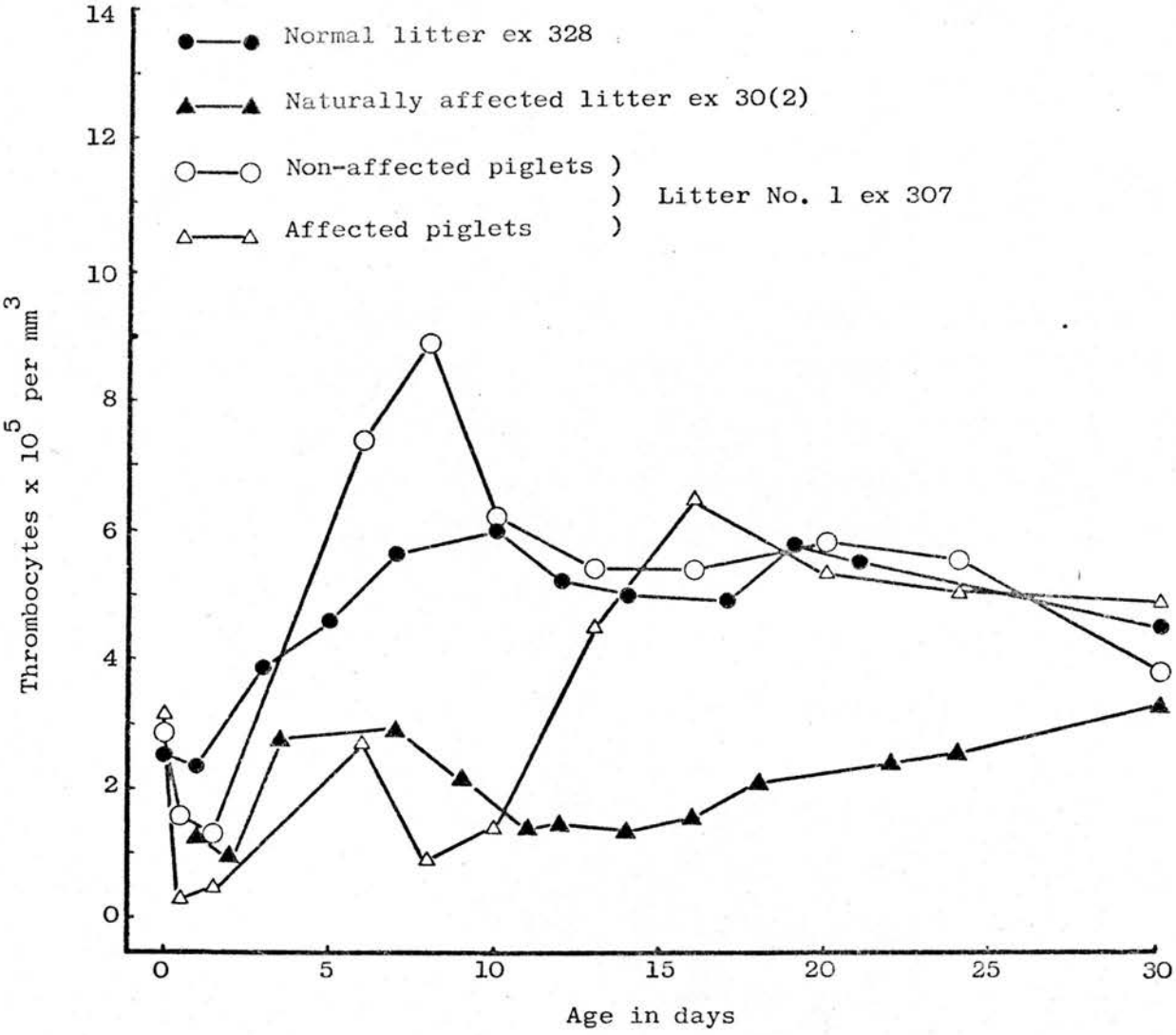

Fig. 6.1 Mean thrombocyte counts of piglets (affected and non-affected with purpura) in first litter from sow 307 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

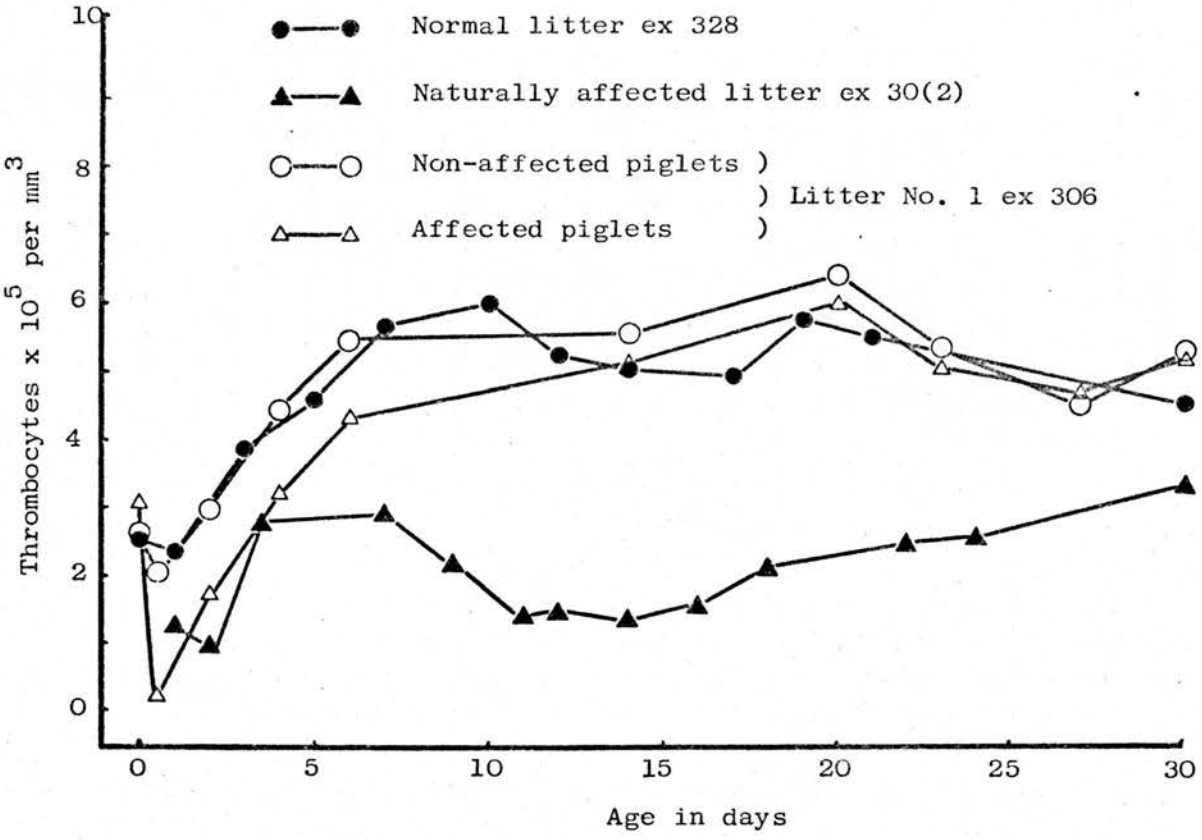

Fig. 6.2 Mean thrombocyte counts of piglets (affected and non-affected with purpura) in first litter from sow 306 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

(b) Repeat mating - no further immunisation

Sow 307 was again mated to the same boar, 311, to see if the antibodies persisted at a high enough level to produce the condition without further artificial stimulation. The piglets were observed clinically and thrombocyte counts carried out at intervals as previously (Appendix VI, Table 5).

Eleven piglets were born, all apparently healthy and with thrombocyte counts within the normal range (Table 6.5). Twenty four hours after ingesting colostrum, five piglets were showing small subcutaneous haemorrhages and the others were apparently normal. There was, however, no significant difference in the mean thrombocyte counts of affected and non-affected piglets at this time. At forty eight hours the haemorrhages were still present, the mean thrombocyte counts of the affected were now significantly lower than the non-affected ones ($0.01 > p > 0.001$). After this time the haemorrhages gradually regressed until they were not seen at five days when there was still a significant difference in mean thrombocyte counts of piglets which had been affected and these which had not ($0.05 > p > 0.01$). From this point on, no more signs of haemorrhages were seen in the piglets and the mean thrombocyte counts of both groups followed a similar course.

When the mean thrombocyte counts of the affected piglets are compared with those of a normal litter (Fig. 6.3), it can be seen that they followed a similar course for the first few days after birth. After five days, however, the naturally affected piglets showed a secondary drop in thrombocytes while the experimentally affected did not.

Table 6.5

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in second litter ex 307 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Difference + S.E.	DF	t	Sig.
Birth	239 ± 11.3 (4)	227 ± 32.6 (4)	12 ± 39.9	6	0.30	NS
24 h	150 ± 15.6 (6)	102 ± 21.0 (5)	48 ± 28.4	9	1.69	NS
48 h	202 ± 15.4 (6)	96 ± 24.8 (5)	106 ± 28.2	9	3.76	**
5 d	426 ± 18.0 (6)	345 ± 18.2 (5)	81 ± 25.8	9	3.156	*
7 d	477 ± 22.0 (6)	470 ± 27.1 (5)	7 ± 34.5	9	0.181	NS
12 d	520 ± 47.8 (6)	489 ± 39.6 (5)	31 ± 63.9	9	0.485	NS
14 d	464 ± 38.1 (6)	507 ± 25.5 (5)	-43 ± 48.0	9	0.906	NS
19 d	504 ± 5.1 (6)	523 ± 11.8 (5)	-19 ± 12.0	9	1.523	NS
21 d	450 ± 15.7 (6)	478 ± 15.5 (5)	-28 ± 22.3	9	1.27	NS
23 d	450 ± 25.5 (6)	448 ± 36.2 (5)	2 ± 42.1	9	0.048	NS
29 d	498 ± 41.5 (6)	485 ± 28.0 (5)	13 ± 52.4	9	0.252	NS
36 d	416 ± 41.8 (6)	428 ± 22.6 (5)	-12 ± 50.5	9	0.241	NS

** - $0.01 > p > 0.001$ * - $0.05 > p > 0.01$ NS - not significant

The non-affected piglets in the experimental litter showed a more marked drop at twenty four hours than the piglets in a normal litter by the same boar and it would seem that their thrombocytes were affected to a certain degree though they showed no symptoms of purpura.

At eight weeks of age the antiglobulin consumption test was carried out using platelets from the piglets and serum from the sow (Appendix 6, Table 6). All the piglets which showed signs of purpura were strongly positive. However, of those which were apparently normal, some gave inconclusive reactions. There was therefore no complete correlation between purpura, thrombocytopenia and the serological reactions of the platelets in this case (Table 6.6).

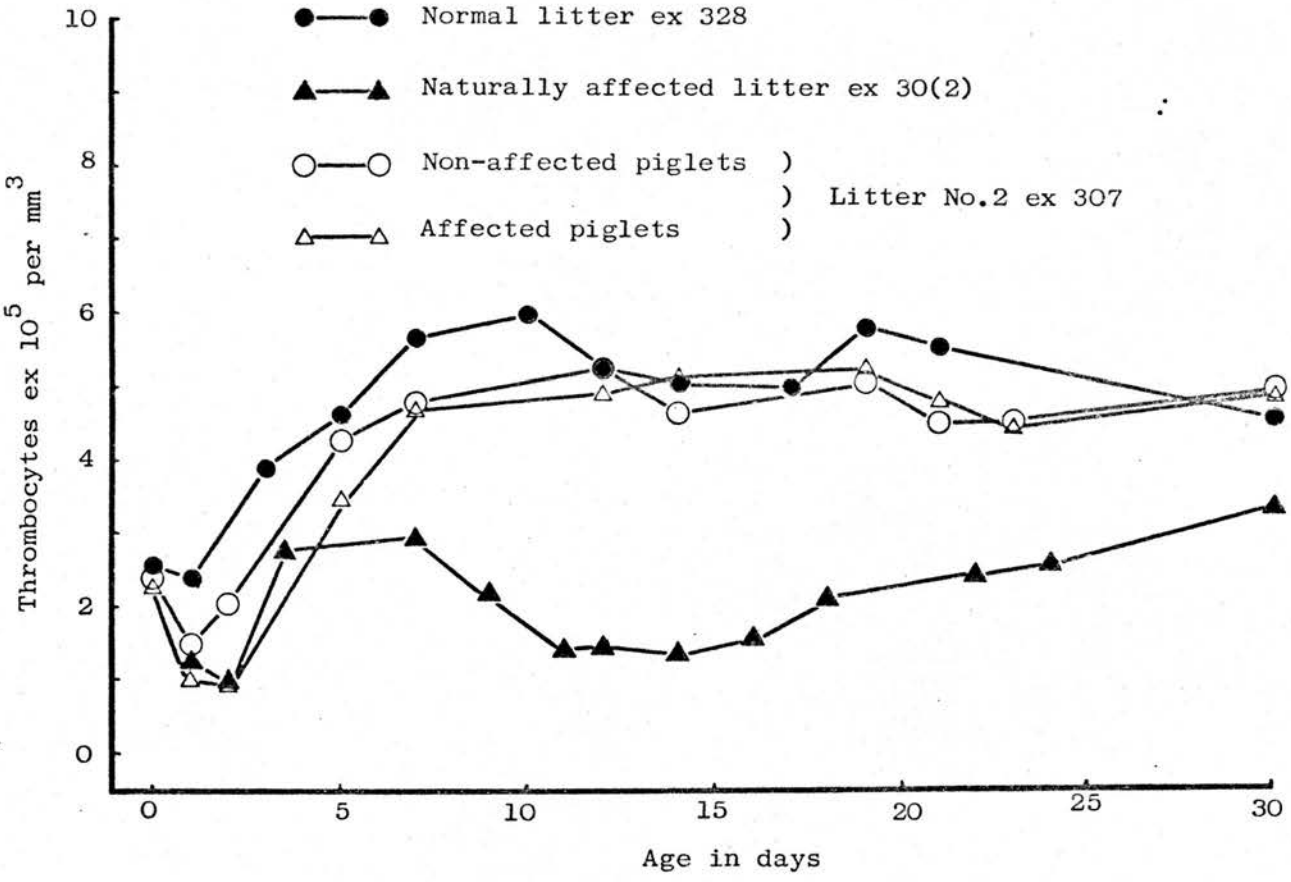

Fig. 6.3 Mean thrombocyte counts of piglets (affected and non-affected with purpura) in second litter from sow 307 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

Table 6.6

Clinical signs and serological reactions of platelets of piglets in second litter ex 307 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
482	+	++	+
483	-	±	-
484	-	±	-
485	+	±	+
486	-	-	-
487	-	±	±
488	+	++	+
489	+	++	+
490	-	±	±
491	+	++	+
492	-	+	±

A.G.C.T. - antiglobulin consumption test.

\+ - positive

\- - negative

± - inconclusive

(c) Effect of restimulating a sow with boar's thrombocytes one week before parturition.

Sow 306 was mated to 311 again and injected with a suspension of thrombocytes from him one week before parturition to see if a secondary thrombocytopenia could be produced.

Ten piglets were born and thrombocyte counts carried out at birth and at intervals up to five weeks of age (Appendix VI, Table 7). Before getting colostrum all piglets were normal and all had thrombocyte counts within the normal range. Twenty four hours later two piglets were still normal, two had been killed by overlaying and six were showing severe signs of purpura. The mean thrombocyte count of the affected piglets was significantly lower than that of the non-affected ones ($p < 0.001$) (Table 6.7). Four of the affected piglets died before the next sampling the following day and the haemorrhages in the two survivors gradually regressed and were not apparent by six days of age. Mean thrombocyte counts of the affected piglets were still significantly lower than the non-affected ones at forty eight hours ($0.01 > p > 0.001$) and at four days ($0.05 > p > 0.01$) but after this time there was no difference between the means throughout the period of study.

When compared with a normal litter (Fig. 6.4) the mean thrombocyte counts of the normal piglets in the experimental litter followed a similar pattern. On the other hand, the affected piglets in the experimental litter were similar to the naturally affected litter only during the first few days of life and did not show the secondary thrombocytopenia of the latter.

The antiglobulin consumption that was carried out in the platelets of the four surviving piglets and serum from the dam (Appendix VI, Table 8). There was complete correlation between the clinical signs of purpura and thrombocytopenia and the serological reactions of the platelets for these animals (Table 6.8).

Table 6.7

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in second litter ex 306 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Difference ± S.E.	DF	t	Sig.
Birth	366 ± 32.5 (2)	268 ± 26.4 (8)	-98 ± 56.6	8	1.73	NS
24 h	274 ± 19.5 (2)	46 ± 10.6 (6)	228 ± 21.4	6	10.65	***
48 h	339 ± 20.6 (2)	112 ± 4.5 (2)	227 ± 21.0	2	10.81	**
4 d	449 ± 23.0 (2)	391 ± 26.0 (2)	58 ± 11.0	2	5.27	*
7 d	531 ± 17.5 (2)	583 ± 28.5 (2)	-52 ± 33.4	2	1.556	NS
9 d	535 ± 65.0 (2)	492 ± 52.0 (2)	43 ± 83.2	2	0.516	NS
11 d	481 ± 28.0 (2)	438 ± 91.0 (2)	43 ± 95.2	2	0.451	NS
15 d	481 ± 86.0 (2)	417 ± 101.0(2)	64 ± 132.6	2	0.482	NS
18 d	443 ± 45.0 (2)	441 ± 25.0 (2)	2 ± 51.5	2	0.038	NS
21 d	472 ± 6.0 (2)	498 ± 38.0 (2)	-26 ± 38.5	2	0.675	NS
24 d	415 ± 3.5 (2)	464 ± 67.0 (2)	-50 ± 67.1	2	0.737	NS
35 d	340 ± 56.5 (2)	311 ± 101.8(2)	29 ± 115.7	2	0.246	NS

*** - $p < 0.001$ ** - $0.01 > p > 0.001$ * - $0.05 > p > 0.01$ NS - not significant

Table 6.8

Clinical signs and serological reactions of platelets of piglets in second litter ex 306 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
493	-	-	-
494	++	++	ND
495	-	-	-
496	++	++	ND
497	-	ND	ND
498	++	++	+
499	++	++	ND
500	-	ND	ND
501	++	++	+
502	++	++	ND

ND - Not done

A.G.C.T. - Antiglobulin consumption test.

\+ - positive

\- - negative

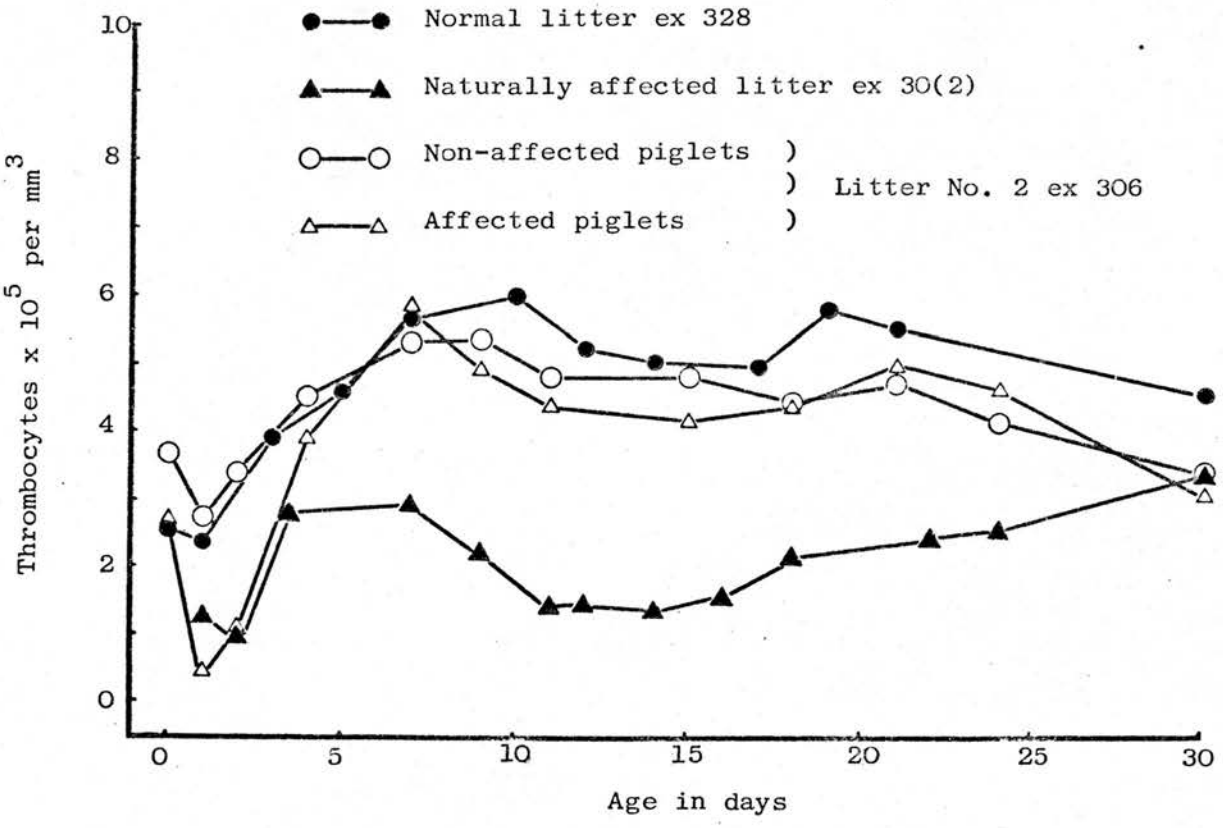

Fig. 6.4 Mean thrombocyte counts of piglets (affected and non-affected with purpura)in second litter from sow 306 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

(d) Effect of restimulating a sow with boar's thrombocytes at parturition.

Sow 307 was mated for a third time with 311 and given a further injection of thrombocyte suspension from that boar at the time of parturition.

Ten normal piglets were born and six were affected with purpura after ingesting colostrum, the other four remaining normal. Thrombocyte counts were carried out on all piglets from forty eight hours of age onwards (Appendix VI, Table 9). At forty eight hours the mean thrombocyte count of the affected piglets was significantly lower than that of the non-affected ones (Table 6.9). By five days, however, there was no significant difference and from eight days onwards both groups followed a similar pattern, there being no secondary thrombocytopenia in the affected piglets.

When compared with a normal litter, the mean thrombocyte counts of the non-affected piglets in this experimental litter followed a similar pattern, but did not reach such high levels (Fig. 6.5). The affected piglets in the experimental litter, on the other hand, followed the naturally affected litter for the first five days but after this they followed their non-affected litter-mates and no secondary thrombocytopenia or purpura was seen as in the naturally affected litter.

The antiglobulin consumption test was carried out on nine surviving piglets at eight weeks of age (Appendix VI, Table 10). All piglets which showed signs of purpura were thrombocytopenic but two of the normals had lower thrombocyte counts than expected (Table 6.10) and this was confirmed by

the serological reactions, all the affected piglets being positive to the antiglobulin consumption test, while one of the normals which had a slight thrombocytopenia was doubtful. The other two surviving normal animals gave a negative result.

Table 6.9

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in third litter ex 307 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Difference ± S.E.	DF	t	Sig.
48 h	188 ± 19.65 (4)	102 ± 15.17 (6)	85.9 ± 24.51	8	3.506	**
5 d	314 ± 20.44 (4)	269 ± 19.90 (6)	45.75 ± 29.65	8	1.543	NS
8 d	454 ± 16.42 (4)	467 ± 30.83 (6)	-12.75 ± 40.66	8	0.313	NS
10 d	435 ± 11.49 (4)	373 ± 24.89 (6)	62.75 ± 42.34	8	1.482	NS
12 d	321 ± 8.66 (3)	324.5 ± 11.77 (6)	-3.5 ± 18.14	7	0.192	NS
15 d	335 ± 3.46 (3)	332.2 ± 27.2 (6)	2.84 ± 39.89	7	0.071	NS
17 d	292 ± 20.5 (3)	298.3 ± 28.03 (6)	-6.7 ± 43.18	7	0.154	NS
19 d	287.7 ± 36.95 (3)	292.7 ± 34.0 (6)	-5.0 ± 55.39	7	0.090	NS
22 d	348.67 ± 12.47 (3)	322 ± 30.14 (6)	26.67 ± 44.87	7	0.594	NS
26 d	343 ± 11.00 (3)	319.5 ± 22.77 (6)	23.5 ± 34.10	7	0.689	NS

** - $0.01 > p > 0.001$ NS - not significant

Table 6.10

Clinical signs and serological reactions of platelets of piglets in third litter ex 307 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
580	-	-	-
581	+	++	+
582	-	-	-
583	+	±	+
584	+	++	+
585	-	±	ND
586	+	+	+
587	-	±	±
588	+	+	+
589	+	+	+

A.G.C.T. - antiglobulin consumption test.
ND - not done
\+ - positive
\- - negative
± - inconclusive

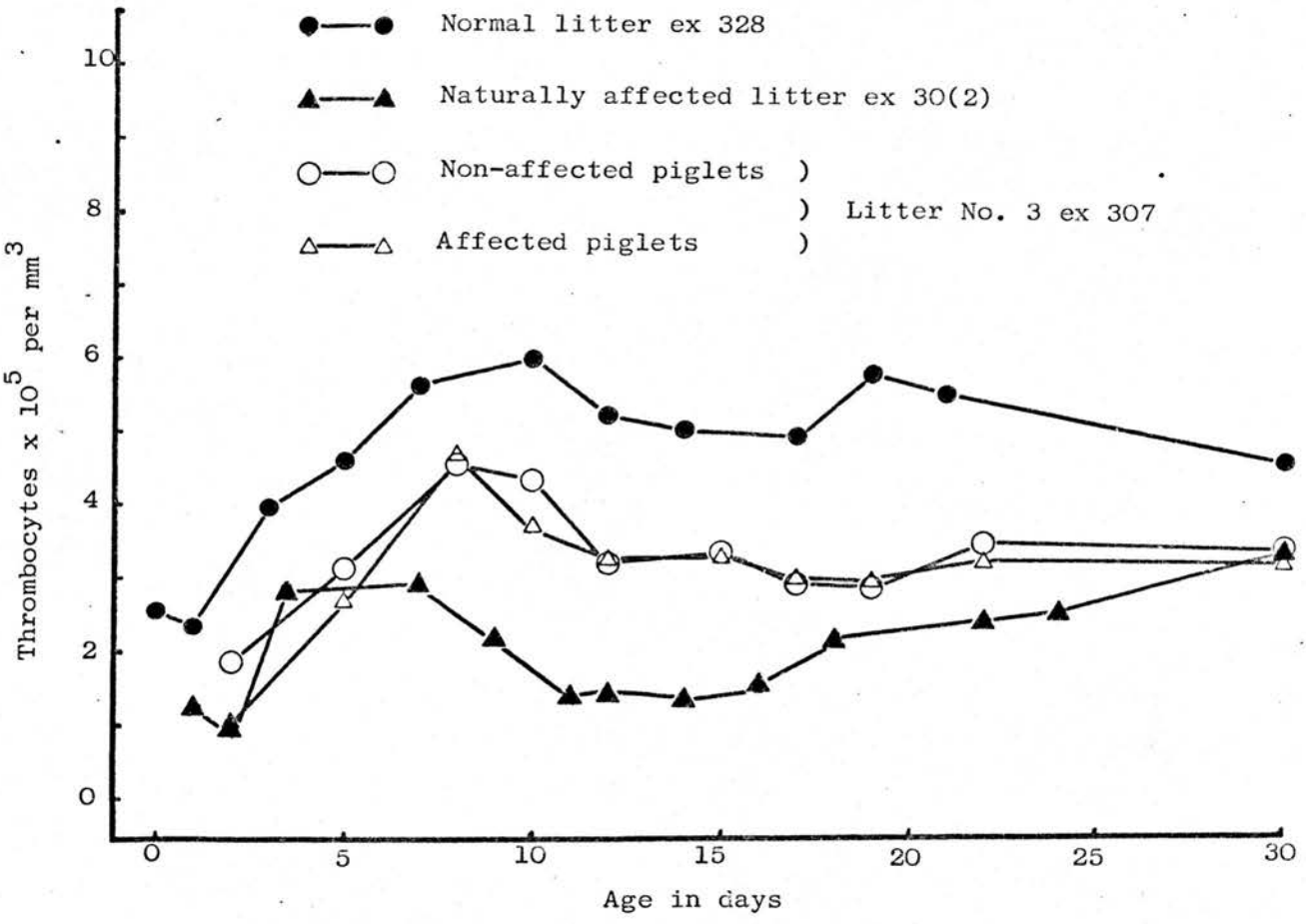

Fig. 6.5 Mean thrombocyte counts of piglets (affected and non-affected with purpura) in third litter from sow 307 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

(e) Effect of restimulating a sow with a suspension of boar's thrombocytes both two weeks before and at parturition.

Sow 306 was mated to boar 311 for a third time and given further injections of thrombocyte suspensions from that boar two weeks before and at parturition.

At birth all nine piglets had normal thrombocyte counts (Table 6.11). All piglets were examined for signs of purpura and further thrombocyte counts carried out at intervals up to twenty five days of age (Appendix VI, Table 11). Within twenty four hours, five piglets were severely affected with purpura, four were still normal. Two were so severely affected that they were not sampled but the mean thrombocyte count of the other three affected ones was significantly lower than that of the normal ones ($p < 0.001$). At three days of age the affected piglets were still showing fairly severe purpura and their mean thrombocyte count was still lower than that of the normal ones ($0.01 > p > 0.001$). By five days the haemorrhages had about disappeared and there was no longer any significant difference in the mean thrombocyte counts of the two groups. From this time on the thrombocyte counts of the piglets which had been affected and those which had been clinically normal closely followed the same pattern and no more differences were seen between them.

When compared with the mean thrombocyte counts of a normal litter by the same boar (Fig. 6.6) the mean thrombocyte counts of the non-affected piglets in the experimental litter

followed a similar pattern but at a lower level. The affected piglets in the experimental litter were again similar to those of the naturally affected litter for the first few days of life but after five days followed that of their non-affected litter-mates and did not show the secondary thrombocytopenia of the naturally affected group.

At eight weeks of age, platelets from all piglets were tested with the dam's serum by means of the antiglobulin consumption test (Appendix VI, Table 12). The serological reactions of the piglets' platelets were then seen to correspond completely with the clinical signs of purpura and thrombocytopenia manifest within the first few days of life.

Table 6.11

Mean thrombocyte counts and standard errors x 10^3 per mm^3 of non-affected and affected piglets in third litter ex 306 by 311 with numbers sampled in brackets.

Age	Non-Affected	Affected	Difference ± S.E.	DF	t	Sig.
Birth	244 ± 16.7 (4)	243 ± 8.8 (5)	1 ± 17.26	7	0.634	NS
24 h	222 ± 3.4 (4)	81 ± 10.5 (3)	140 ± 9.67	5	14.49	***
3 d	347 ± 12.5 (4)	187 ± 25.7 (4)	160.5 ± 28.54	6	5.623	**
5 d	357 ± 23.6 (4)	357 ± 22.5 (5)	0.1 ± 32.92	7	0.018	NS
7 d	508 ± 17.3 (4)	526 ± 23.1 (5)	-17.6 ±30.275	7	0.581	NS
10 d	443 ± 38.8 (4)	371 ± 38.8 (5)	71.7 ± 55.6	7	1.289	NS
12 d	341 ± 20.7 (4)	337 ± 33.5 (5)	4.4 ± 42.05	7	0.104	NS
14 d	360 ± 6.7 (4)	334 ± 37.5 (5)	26.1 ± 42.90	7	0.608	NS
17 d	369 ± 6.4 (3)	366 ± 37.7 (5)	3.1 ± 42.98	7	0.072	NS
19 d	397 ± 22.1 (4)	350 ± 40.9 (5)	46.7 ± 50.34	7	0.927	NS
21 d	348 ± 38.0 (4)	354 ± 40.2 (5)	-5.8 ± 56.51	7	0.102	NS
25 d	405 ± 37.1 (4)	385 ± 27.1 (5)	19.7 ± 47.79	7	0.412	NS

*** - $p < 0.001$ ** - $0.01 > p > 0.001$ NS - not significant

Table 6.12

Clinical signs and serological reactions of platelets of piglets in third litter ex 306 by 311.

Piglet No.	Purpura	Thrombocytopenia	A.G.C.T.
563	-	-	-
564	+	+	+
565	++	ND	+
566	-	-	-
567	-	-	-
568	-	-	-
569	++	ND	+
570	+	+	+
571	+	+	+

A.G.C.T. - antiglobulin consumption test.
ND - not done
\+ - positive
\- - negative

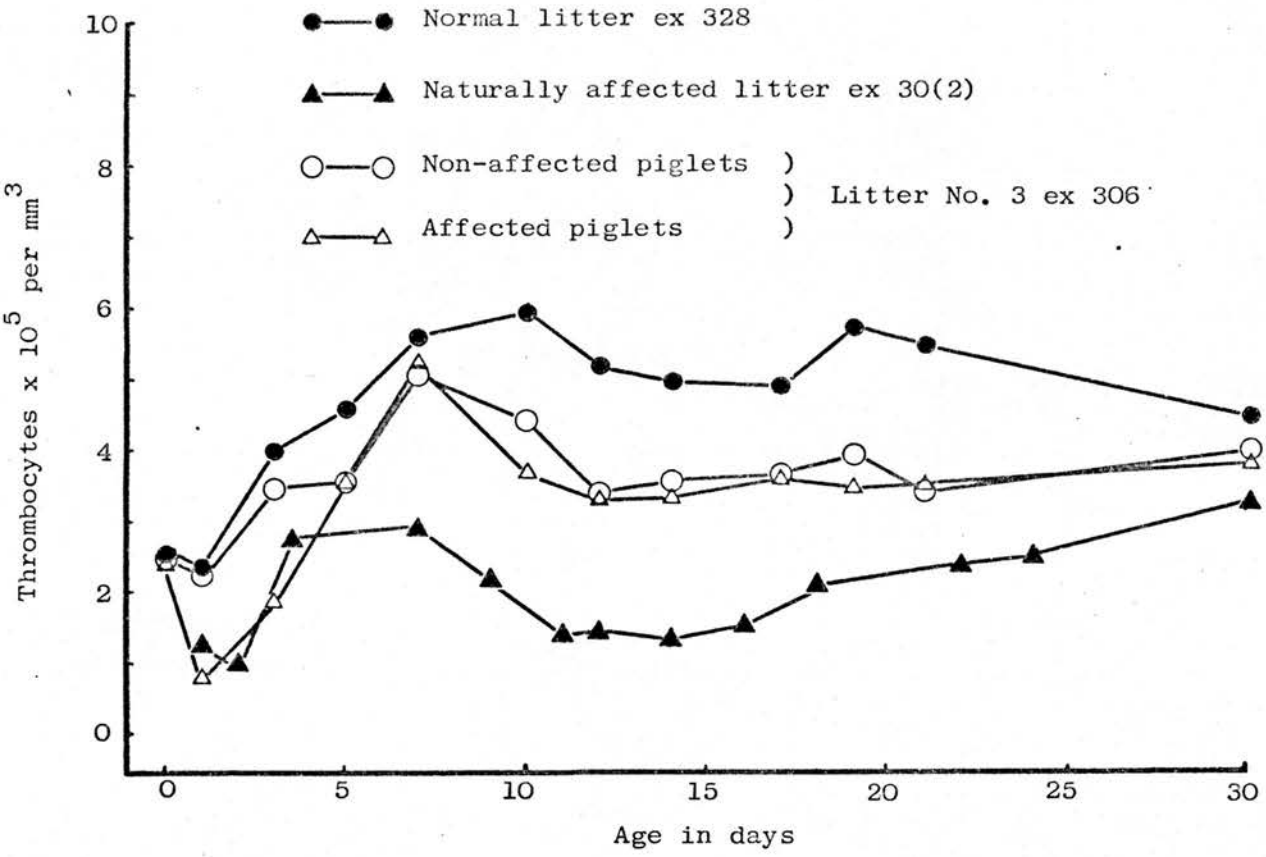

Fig. 6.6 Mean thrombocyte counts of piglets (affected and non-affected with purpura) in third litter from sow 306 as compared with those of a normal litter (sow 328) and of a litter naturally affected with thrombocytopenic purpura (second litter from sow 30).

Conclusions

A condition manifested by thrombocytopenia and subsequent purpura was experimentally produced in piglets by injecting their dams with thrombocyte suspensions from their sire before parturition. Signs appeared after the piglets ingested the anti thrombocyte antibodies in the sow's colostrum.

The signs both in live piglets and at necropsy (Plates 6.1 & 6.2) were similar to those found in naturally affected piglets (Plates 5.1 and 5.2). Thrombocyte counts of the former were similar to those of the latter within the first few days of life but only in one litter, i.e. the first litter from sow 307, was the secondary thrombocytopenia, characteristic of piglets in naturally affected litters, observed. As the anti thrombocyte antibody level in sow 307 was boosted within ten days of parturition in this case and the piglets were very severely affected, it was thought that further stimulation at this time might be involved in the production of the secondary thrombocytopenia. However, two further attempts at this (6.c and 6.e) failed.

Similarly, attempts at restimulating the antibody levels of the sows at parturition failed to produce a secondary thrombocytopenia (6.d and 6.e).

That the anti thrombocyte antibodies could be maintained at a sufficiently high level in the sow to produce clinical signs of purpura and thrombocytopenia at a subsequent parturition without any further artificial stimulation was also shown (6.b). However, the piglets born at this

parturition were not as severely affected as those of the previous one indicating some drop in the antibody titre.

In all litters of both sows, both affected and non-affected piglets were present. In all three litters from 306 there was a clearcut division, the non-affected piglets having thrombocyte counts similar to normal piglets and there was a complete correlation between the clinical signs of purpura and thrombocytopenia and the reactions of the piglets thrombocytes with their dam's serum (antiglobulin consumption test). There were eighteen affected and eight non-affected piglets in the three litters and this supported the theory that the sire was heterozygous for the platelet factor involved while the sow was completely negative for it ($0.05 > p > 0.01$).

In the litters from sow 307 on the other hand, although the piglets could be divided into affected and non-affected by signs of purpura, some of the latter group showed a slight thrombocytopenia. This was followed by the reactions of the piglets' platelets with their dam's serum (antiglobulin consumption test), not completely agreeing with the clinical signs. Therefore it seemed in this case that more than one antibody was produced in the dam and hence the segregation of the piglets into affected and non-affected groups was not clearcut.

D I S C U S S I O N

Red Cell Study

Using the blood group factor Ea, it has been demonstrated that iso-immunisation can occur in sows to foetal antigens which they themselves do not have. It would seem from the limited number of animals studied that the most likely time for this to take place is at parturition as, in three of the sows, it was after this that the rise in anti-Ea titre was noted. Similarly, in the two animals which produced anti-Ea after being mated to an Ea positive boar, it was after parturition that this antibody was first seen. Hojny and Hala (1964) and Cop (1969) have suggested that iso-immunisation of sows can take place as a result of pregnancy but offer little direct evidence to support their theory. The data presented here is the first recorded evidence for this occurrence.

As well as Szent-Ivanyi and Szabo (1954) and Goodwin, Saison and Coombs (1955), several other workers have found red cell antibodies other than anti-A in the serum of pigs and which could not have resulted from vaccination. Andresen and Wroblewski (1961) found anti-Ka in an immune serum which could not have been produced against donor erythrocytes and Hala and Hojny (1962) often found anti-Ea in normal sera. More recently Cop and Koops (1969) showed that anti-Ea was more common in the sera of sows mated to Ea positive boars than in the sera of those mated to Ea negative boars. However, they had the disadvantage of working with animals which had been vaccinated with crystal violet swine fever vaccine.

In this investigation the possibility of injection with porcine erythrocytes in any form can be completely excluded in the animals in the minimal disease piggery and also, as far as could be ascertained from detailed histories, in those kept under conventinal conditions in the blood group research unit piggery. No histories were available for the other animals but all were of such an age as not to have been vaccinated with crystal violet swine fever vaccine.

As well as being studied most in the experimental sows, anti-Ea is also the antibody which was found most frequently in the survey of the sera sent in for parentage checking, and in the dams of thrombocytopenic litters. This agrees with the findings of Cop and Koops (1969) who found anti-Ea present in fourteen out of eighteen sera which contained iso-antibodies. They were of the opinion that most of these had been formed after pregnancies to Ea positive boars and also reported finding anti-Eb, -Kb and -Kd. In general, the antibodies reported here and by other workers elsewhere are to those factors which are most strongly antigenic in artificial iso-immunisation.

The fact that approximately twenty five per cent of the sera from the sows in the routine typing service showed red cell antibodies other than anti-A, while the sera from the boars did not contain any other than anti-A is further evidence that iso-immunisation by foetal red cells is occurring. This figure is surprisingly high as all the sows were bred to boars of the same breed as themselves and most of them were young animals being blood sampled at four to six weeks after their

first or second parturitions. Due to variations in gene frequencies of red cell factors between different breeds, a higher incidence might be expected where cross-breeding is practised and this has been shown by the fact that the sera of fifty per cent of sows which had produced litters suffering from thrombocytopenic purpura contained antibodies other than anti-A, where most of the sows had been mated to boars of a different breed from themselves.

Hardy (1970) has also reported finding iso-antibodies in pigs without apparent artificial stimulation. However, she is of the opinion that they can occur "naturally", i.e. not following incompatible mating or immunisation, or non specifically following immunisation with other red cell antigens. Out of twenty nine antibodies reported, anti-Ea and anti-Eb were most frequently found by her as well as anti-La and anti-Lg. Ten of the antibodies found were unidentified and no titres were given for any of the antibodies except one (see below). It is therefore significant to note that seven boars in the present investigation did have antibodies present in their sera (Appendix II, Table Ib), but at very low levels (viz. less than fifty per cent reaction at equal volume of neat serum and red cell suspensions). It was, however, found to be impossible to identify these by the usual methods of absorptions and checkerboard analyses and any such reactions were ignored in this investigation. As Hardy was unable to identify such a high percentage of the antibodies she found, it is likely that they were also present at very low levels.

In Hardy's report at least one of the antibodies, anti-Eb, occurred in a hog at ten months of age and at a titre of 1 in 4. Despite the fact that anti-Ea in Ea negative piglets was observed for up to sixteen weeks in this investigation (Results 3), it is unlikely that maternally derived antibody could last for ten months. Although the method studied here is iso-immunisation of pregnancy, this does not preclude that there could be other ways for iso-antibodies to occur. In such cases, iso-immunisation of pregnancy might help to boost the antibody levels.

The question arises as to the significance of these antibodies. In the sows themselves they are obviously of no consequence. It is only when they are secreted in the colostrum and ingested by piglets whose red cells carry the corresponding antigen or antigens that they are likely to be of importance.

Hardy and Shaw (1970) have pointed out that maternally derived anti-red cell antibodies may lead to errors in red cell typing of piglets. Obviously the piglets' red cells, if coated with maternal antibody, will give positive reaction in the indirect sensitisation test and this is the basis of the direct sensitisation (Coombs) test for the diagnosis of haemolytic disease of the newborn. However, Hardy and Shaw also found discrepancies in the direct agglutination test and they showed <u>in vitro</u> that a "naturally occurring" anti-Eb blocked the actions of an Eb reagent. Pigs which were Ee/Eb were typed as Ee/Ee. Where the pigs were homozygous for Eb,

inhibition was still present but to a lesser degree. In this latter case, the genotypes of the animals when typed with the other reagents of the E system would make one suspicious that there was a discrepancy in the typing. Whether these remarks apply to other systems remains to be seen. In the present investigation, the red cells of all piglets gave negative reactions to the direct Coombs test after seven days of age and therefore the errors are not likely to occur after this time. As only piglets which are typed by the direct agglutination technique after receiving colostrum and before one week of age are likely to be affected in this way, this phenomenon is not of much significance.

By far the most important aspect of the effect on piglets is the possibility of the production of haemolytic disease of the newborn and it was this which initially stimulated interest in blood groups in pigs. The part played by crystal violet vaccination for swine fever is beyond dispute but, as this practice has now been abandoned in this country, it is now no longer of significance. On the other hand as demonstrated in this study, iso-immunisation of sows by foetal red cells has been shown to take place and therefore the possibility of this playing a part in the pathogenesis of haemolytic disease of newborn piglets must be considered.

Anti-Ea was the main antibody present in the sera of the dams of the four litters studied. If the criterion for a positive diagnosis, viz. a positive direct Coombs test on the piglets red cells after ingesting colostrum, advocated by Goodwin (1957) is adopted, then these four litters were affected

with haemolytic disease of the newborn. Most of the Ea positive piglets red cells were positive for this test after ingesting their dams' colostrum and some of them remained positive up to a week of age. All the anti-Ea was apparently taken up by the red cells as none was detected in the serum of these animals, unlike the Ea negative ones, in whose serum anti-Ea could be detected for up to sixteen weeks of age. This latter finding is of interest as it appears that the length of time the maternally derived antibody remains in the piglets' circulation is greatly influenced by whether it comes in contact with the corresponding antigen or not. The reduction in titre coincides with the growth of the piglet and the main effect would appear to be one of dilution in the circulation. The normal metabolism of the piglet did not appear to influence the titre.

Despite the fact that the anti-Ea was taken up by the red cells of Ea positive piglets, it had little effect on them. No difference could be demonstrated in the red cell counts, haemoglobin levels and packed cell volumes between Ea positive and Ea negative litter-mates. This was the case in all litters, even the one from sow 301 which had the highest antibody level at parturition, the titre having been boosted four to six weeks previously.

Cop and Koops (1969) remarked on the fact that, although twenty five per cent of the sows they examined had red cell iso-antibodies in their sera, the piglets did not seem to be clinically affected and they put forward a theory that a complement factor might be lacking in piglets. Pigs have low

levels of haemolytic complement in their sera (Coombs, Coombs and Ingram, 1961) and this may well protect them from the effects of the red cell iso-antibodies.

Further, the individual factor or factors involved are likely to be important. As well as those implicated in haemolytic disease of newborn piglets by Joysey, Goodwin and Coombs (1959a), viz. Fa, Gb, and Lg, and by Andresen and Baker (1963), viz. Ba, several other workers have listed factors which they found to be involved in clinical cases. Cop (1969) reported four cases due to anti-Fa, one due to a factor of the L system and one probably due to anti-Ea while Mayer, Rasmusen and Simon (1969) found anti-Ba, anti-Fa and anti-Kb in four cases. The latter report is interesting in that it is claimed that iso-immunisation had occurred without artificial stimulation, i.e. after previous pregnancies, but the sows had been vaccinated with live virus hog cholera vaccine and given antiserum simultaneously. Although not as likely as with the crystal violet vaccine, there is still the possibility that the live virus hog cholera vaccine and the antiserum could have been contaminated with porcine antigens and could have given rise to the antibodies described. In the cases reported from Japan, one factor was thought to be involved as it was present on the red cells of all affected piglets and their sire (Himeno, Nagano, Mogi, Abe and Hosoda, 1969) but these workers were not able to translate it into present-day nomenclature when comparing with the reagents of the Iowa State University and called it S7.

It is therefore significant that the antibodies most

frequently found, i.e. anti-Ea and anti-Eb, are seldom incriminated in haemolytic disease of newborn piglets, there being only one report of anti-Ea having possibly been involved. Talbot and Andresen (1964) found that anti-Ea greatly reduced the half-life of Ea positive erythrocytes transferred into piglets and suggested that this antibody might be important in haemolytic disease of the newborn especially as it is so easily produced in incompatible transfusions. However, this does not seem to be so in newborn piglets. In general, the factors of the E System are those to which iso-antibodies are easily produced in pigs and they tend to give strong direct agglutinating reactions. For haemolytic disease of newborn piglets to be induced naturally the important incompatible factors would seem to be Ba, Fa, Gb, Kb and Lg.

At the same time it is relevant to consider the gene frequencies of these factors in the main two breeds, Large White and Landrace, used for cross-breeding in this country. As Ba is of very high frequency in both breeds it is unlikely to be of significance. With regard to the other factors, it is useful to consider the work of Gavalier, Hojny, Hradecky, Linhart and Schroffel (1966) who compared the gene frequencies of red cell factors in pigs of several breeds including Landrace and Large White. Statistically ($p < 0.01$) they showed that Fa, Gb and Lg were more common in Landrace than Large White while Kb was more common in Large White than Landrace. Hence, haemolytic disease of the newborn due to anti-Fa, anti-Gb or anti-Lg would be more likely to occur in

the litters of Large White sows by Landrace boars while anti-Kb would be more likely to be involved in the disease in litters of the reciprocal matings. In matings of the first cross sows back to boars of either of the parent breeds, as is frequently practiced commercially, the possibility for the occurrence of haemolytic disease in the litters produced would again be reduced though not to as low a level as in pure-bred matings.

As well as the factors involved, the level of antibody present in the dams' serum and colostrum must play a part. In the cases reported by Goodwin, Heard, Hayward and Roberts (1956), serum titres in the sows of 1 in 256 or less by the indirect sensitisation test produced only mild symptoms of haemolytic anaemia in newborn piglets after ingesting their dams' colostrum. It was not until serum titres of 1 in 2048, by the same technique, were present in the dams' serum that severe symptoms were present in their piglets. The observation in the present study that the antibody titre in most of the animals studied had waned to 1 in 16 or less by the following parturition meant that the piglets were protected from absorbing high levels of red cell antibodies via the colostrum. Goodwin and Saison (1957) noted two types of response to crystal violet vaccination. In some animals they found the red cell antibody level was maintained at a high level for a long time while in others it gradually tailed off. In this investigation it is interesting to note that a rise in red cell iso-antibody titre occurred in one sow four to six weeks before parturition. If this occurred more commonly,

this would obviously be a way in which haemolytic disease of newborn piglets might be produced as the titre was still high at parturition. However, anti-Ea which was the main antibody present in the litter studied and which was present in the sow's serum at farrowing at a titre of 1 in 128 by the indirect sensitisation technique, had little effect on the piglets' red cells.

Apart from the titre, the character of the antibody or antibodies present is likely to be of importance. Complement dependent (i.e. haemolytic) ones and/or incomplete agglutinins (i.e. those detected by the indirect sensitisation test) may be more important than complete agglutinins (i.e. those detected by the direct agglutination test). Of blood typing reagents in pigs, few are haemolytic and most are complete or incomplete agglutinins. Very few complete agglutinins go beyond titres of 1 in 64 to 1 in 128 whereas indirect agglutinins and haemolytic antibodies often exceed 1 in 1000. In this context it is pertinent to note that the Rh factor in humans was most successfully demonstrated by the indirect sensitisation test (Coombs, Mourant and Race, 1954). It may therefore be that, while anti-Ea which is usually present as a strong complete agglutinin was easily produced in the sows studied and acted as a good indicator for the occurrence of iso-immunisation of pregnancy, it may be of no consequence in the pathogenesis of haemolytic disease of newborn piglets.

Hence, for piglets to be affected with haemolytic disease of the newborn, it would seem that several events must take place.

1. The sow must be mated to a boar carrying incompatible red cell antigens for at least two pregnancies in succession, possibly more. The chances of this occurring are obviously higher where cross-breeding is practised.
2. Iso-immunisation must occur. Although incompatible matings may be quite common, iso-immunisation does not occur in every case.
3. The sow must be one which maintains a high antibody titre from one parturition to the next. Alternatively, iso-immunisation must occur in the late stages of pregnancy, so that the piglets are exposed to a high antibody level in the colostrum.
4. Some red cell factors are more likely to be involved in the pathogenesis of haemolytic disease of the newborn than others. Therefore the incompatible factor or factors involved are important.
5. The character of the antibody response induced may be important. Haemolytic antibodies and incomplete agglutinins may be more involved than complete agglutinins.

The chances of all these occurring in a sow are obviously small and this presumably accounts for the fact that clinical haemolytic disease of newborn piglets following iso-immunisation of pregnancy has not as yet been recorded. All cases reported so far have occurred in sows showing a history of immunisation by injections, either of biological products contaminated with red cell antigens or of red cell themselves.

Thrombocyte Study

On turning to the thrombocyte investigations, the picture is less clear. The antiglobulin consumption test, while being the most reliable, still has many faults. As it is very time-consuming, it is not suitable as a routine test. Further, as it was found that, in order to control the test accurately, a preimmunisation sample of serum had to be available for comparison with the reaction of the respective immune one, it is not suitable for testing unknown sera, e.g. those from the dams of thrombocytopenic litters, for the presence of thrombocyte iso-antibodies. Neither is it suitable for titrating the antibody level in a serum sample.

The lack of success with the complement fixation technique is surprising as, despite the disadvantage of the procomplementary effect of inactivated porcine serum, it has been found to be useful in the detection of other antigen-antibody reactions in porcine serology, e.g. the serological diagnosis of mycoplasmal infections in pigs. For this, Roberts (1968) used 1.125 units of guinea pig complement and the porcine serum was not inactivated either by formalin treatment or by heating at 56°C for 30 min. Similarly Hodges and Betts (1969) did not inactivate the porcine serum when using a similar system while Wallis and Thompson (1969) found that inactivating the porcine serum either by heat or formalin, removed the complement-fixing antibodies. On the other hand, Cowan (1961) satisfactorily demonstrated complement fixing antibodies to African swine fever virus in pigs which had recovered from the infection, by removing the procomplementary effect of heated porcine serum

with formalin. Later (Cowan, 1963) he showed that normal bovine serum enhanced the complement fixation reaction in the same system. Hence it would seem that if the complement fixation test is to be studied further, either it should be carried out with porcine serum which has not been inactivated by formalin or heating or normal bovine serum should be added in an attempt to enhance the reaction.

However, it may be more profitable to examine the conglutinating complement absorption test. Porcine serum has higher levels of conglutinating complement than haemolytic complement (Coombs, Coombs and Ingram, 1961) and the conglutinating complement absorption test has been used successfully for the detection of antibodies in porcine serum in other systems. Taffs (1963) found this test more sensitive than the haemolytic complement fixation test for the detection of antibodies to Ascaris suum in porcine sera which had been inactivated by heating at 56°C for thirty minutes before the procomplementary activity was removed by the method of Wiggin (1955). This involved absorbing the porcine sera with washed, packed sheep red cells before treating with dilute hydrochloric acid to remove the resulting anticomplementary activity.

When considering these results it is worth noting that all have been involved in the detection of reactions between porcine antibodies and hetero-antigens. In the investigation of suitable techniques for the detection of thrombocyte antigen-antibody reactions (Results 4), a similar system was used, viz. bovine thrombocytes and porcine antiserum, and the fluorescent antibody, immuno-diffusion and complement fixation techniques

were found to detect reactions between the porcine serum and the hetero-antigen. Similar results were found for the converse system, porcine platelets and rabbit antisera. Therefore, as these results could not be translated into the iso-antibody/iso-antigen system, viz. porcine thrombocytes and porcine antiserum, care must be exercised in drawing too many parallels between hetero antigen-antibody systems and iso antigen-antibody systems in pig serology. Techniques suitable for the detection of the former are not necessarily of use for the latter.

The lack of a suitable serological technique for the detection of porcine thrombocyte antigen-antibody reactions has hampered the studies on thrombocytopenic purpura in piglets. It has not been possible to demonstrate an increase in antibody levels in the sows' sera after farrowing as in the case of the red cell antigens and also to definitely show that the sows'sera contain antibodies to the thrombocytes of affected piglets. However, the fact that fifty per cent of the dams had red cell iso-antibodies in their sera as compared to about twenty-five per cent of the sows in the routine samples is evidence that iso-immunisation by foetal cells is taking place and is more common than in the dams of non-affected piglets. That red cell iso-antibodies are not produced in all cases may merely be due to the fact that, while the thrombocytes of the foetuses may be incompatible with the dam in all cases, the foetal red cells are not necessarily always incompatible with the dam. Vaccination with crystal violet swine fever vaccine could be precluded in all but two

of the dams of the thrombocytopenic litters, one of which had a red cell iso-antibody present in the serum, the other of which had not.

The condition of thrombocytopenic purpura in piglets has been described in detail by Lie (1968) in Norway, by Saunders and Kinch (1968) in Cambridge and more recently by Nansen, Nielsen and Nielsen (1970) in Denmark. The occurrence and symptoms as described in all these cases are similar to that found here (Results 5). Piglets are normal when born but develop thrombocytopenia after ingesting colostrum from the dam. In most cases a secondary thrombocytopenia occurs in the survivors at from ten to fourteen days of life though Lie found some litters in which this did not occur. Purpura with or without subsequent death may occur at either or both times but the detection of thrombocytopenia is the most characteristic and consistent finding.

The primary thrombocytopenia occurring after ingesting colostrum agrees with the iso-immunisation theory and is the symptom which is easily reproduced in the experimental litters (Results 6). On the other hand, the secondary thrombocytopenia is more difficult to comprehend and was only seen in two piglets in one of the experimental litters. Attempts to reproduce this in the other litters by artificially stimulating the sows with thrombocyte suspensions from the boar at various times before and around parturition failed.

Lie is of the opinion that the secondary thrombocytopenia is due to an effect of the antibody on the bone marrow, inhibiting the production of megakaryocytes, but not knocking

out those already formed. However, that this did not occur in most of the experimental piglets tends to invalidate her theory and to suggest that the naturally occurring condition is not simply brought on by iso-immunisation alone. The cross-suckling experiments carried out here and by Saunders and Kinch, definitely point to the ingestion of the dam's colostrum being involved and the most likely cause of the primary thrombocytopenia in the piglets is the absorption of an anti-thrombocyte antibody from this.

The question therefore still centres on how the secondary thrombocytopenia occurs and fresh hypotheses for this have to be sought. As the time of its onset coincides with the time it would take the piglet to produce its own antibodies, the possibility of an auto-immune reaction being involved arises, in which case the piglets may form an antibody to the complex formed by its own thrombocytes and the maternally-derived antibody. As the latter may be incompatible to the piglet, i.e. the piglet is capable of producing an allotype to its dam's antibody, the auto-immune phenomenon may only arise to that complex and not to the one formed by its own antibodies and thrombocytes at ten to fourteen days of age at the time of the secondary thrombocytopenia. Hence the condition does not recur in the survivors after this time. The theory can then be put forward, that, in the limited number of sows in the experimental cases, all the piglets were of a similar allotype to their dams and did not produce antibodies to the complex formed by their own thrombocytes and their dam's antibodies.

Several other factors are known to cause thrombocytopenia in humans and other animals and these are worthy of comment as far as this condition in pigs is concerned. There are agents which act directly on the platelets causing thrombocytopenia such as various viral and bacterial infections. In dogs, thrombocytopenia is associated with the viraemic stage of contagious canine hepatitis and this may be an example of platelets phagocytising viruses as part of the body defence mechanisms (Danon, Jerushalmy and De Vries, 1959). Gram negative bacterial infections can be associated with thrombocytopenia (Cohen and Gardiner, 1966) and, as these infections are common in pigs, it is conceivable that thrombocytopenia could be present as a complication. However, Saunders and Kinch (1968) were unable to find any evidence of bacterial or viral infections in their cases and, in general, thrombocytopenic purpura in piglets does not follow the course of an infectious disease, cases occurring very sporadically.

As platelets may phagocytose antigen-antibody complexes (Movat, Weiser, Glynn and Mustard, 1965), this is another way in which thrombocytopenia may be produced although no examples are, as yet, available in pigs. Hypersensitivity to drugs is also a cause of thrombocytopenic purpura in humans and a large number of compounds have been incriminated (Ackroyd, 1958). Saunders and Kinch (1968) considered this possibility but found no evidence to point to any drugs being involved in their cases.

Further studies on thrombocytopenic purpura in piglets should consider these aspects discussed. The search is still

for a better serological test for the detection of thrombocyte antigen-antibody reactions other than the antiglobulin consumption one. As regards the secondary thrombocytopenia, attention should be focused on the bone marrow and the megakaryocytes, from birth up to fourteen days of age.

It has been shown that iso-antibodies to the antigens on porcine platelets can be formed. These were detected both *in vitro* by means of the antiglobulin consumption test and *in vivo* by the thrombocytopenia and purpura produced in piglets after ingesting their dams' colostrum. The latter also demonstrated that these iso-antigens were inherited as the antibodies were produced in the dam's sera by the parentral injection of thrombocyte suspension from the sire. In all experimental litters (Results 6) both affected and non-affected piglets were present which suggested that the sire was heterozygous for the platelet factor involved, especially in the litters from one of the sows where the clinical and serological findings were in complete agreement. In the litters from the other sow, the picture was not so clearcut and it seemed that antibodies had been produced to more than one factor in this animal. Not enough animals were studied to come to any conclusion about this or get any idea of the frequencies of the factor or factors involved.

With regard to future work, the significance of this part is most likely to be in further elucidation of thrombocytopenic purpura in piglets. The other possibility is that of typing pigs for identification and parentage checking but

this would hardly seem to be feasible. Typing according to red cell antigens and serum proteins as carried out at present would seem to be much superior. The techniques involved lend themselves more easily to routine practices and the increase in exclusion rates which might be brought about by typing platelets would hardly seem to warrant the effort to get reliable reagents. The production of the latter for platelets is not likely to be easy if platelet iso-antibodies in pigs are similar to human ones (Svejgaard and Kismeyer-Nielsen, 1968). Obviously it is also difficult to obtain sufficient quantities of platelets to absorb the volume of serum required for production of stocks of reagents.

Hence, the further elucidation of platelet factors, their frequencies in various breeds and their mode of inheritance would seem to be justified only for the possible prevention of incompatible matings which may result in iso-immunisation during pregnancy and subsequent cases of thrombocytopenic purpura in piglets. It is likely that, as cross-breeding becomes more common in pigs, so the incidence of this condition is likely to increase. If the main factor or factors involved were known, it would be possible to avoid the matings likely to lead to clinical cases.

Addendum

What seems to be a case of haemolytic disease of newborn piglets following iso-immunisation of pregnancy has just been referred for investigation by the Veterinary Investigation Centre at Cambridge (Hall, 1971).

A Landrace sow which had already produced three litters to the same boar farrowed fourteen piglets to a closely related boar. On the second and third days, all the piglets were seen to be jaundiced. Seven died and two were examined post mortem when they showed signs of severe anaemia and jaundice. At eighteen days of age a further piglet died showing typical signs of thrombocytopenic purpura.

Three piglets from the litter which had been removed from the sow and had not ingested their dam's colostrum were returned at four days of age. These piglets did not become jaundiced and continued to thrive normally after being returned to their dam as did two piglets from another sow by the same boar.

No haematological examinations were carried out in the piglets but the sow's serum was tested for antibodies to the boar's red cells with the following results.

Technique	Titre
Direct agglutination	1:256
Indirect sensitisation	1:4096
Haemolytic	1:1024

As the boar's red cells were Ao, anti-A did not confuse the picture and these titres are for red cell iso-antibodies. The factors involved were found to be Ea, Gb and Kb.

Both boars with which the sow had been served were found to be homozygous for the factors Gb and Kb, and heterozygous for the E system (Eaeg/Eedg and Eaeg/bdg), i.e. for the factors which the sow lacked.

The conclusion was therefore that this was a case of

haemolytic disease of the newborn. As no artificial means of immunisation could be found from a careful examination of the sow's history, it is suggested that this is a case following iso-immunisation of pregnancy. As a piglet died showing symptoms similar to that of thrombocytopenic purpura, it is possible that iso-immunisation to the foetal thrombocytes had also occurred.

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APPENDIX I

Appendix I

Table I.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of five animals in serial serum samples from 88 during pregnancy and after parturition when mated to (1) an Ea negative boar (Parturition at week 0) followed by (2) an Ea positive boar, BPM2 (Parturition at Week 24).

Test Cell Donor		W3184		71P16		T38P12		BPM2		P20	
Relevant Factors (Antigens)		Aa Ea		Aa -		Ao Ea		Ao Ea		Ao -	
Serological Test		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	- 4	1.71	3.00	1.33	1.50	1.33	1.50	-	-	0.00	0.00
	- 2	1.71	3.00	1.33	1.50	1.00	1.50	-	-	0.00	0.00
	0	1.50	1.50	1.33	1.50	1.71	1.00	-	-	0.00	0.00
	2½	1.50	1.50	1.33	1.50	1.00	1.50	-	-	0.00	0.00
	6	1.71	1.50	1.33	1.71	1.71	1.50	-	-	0.00	0.00
	8	1.50	1.33	1.33	3.00	1.50	1.00	1.50	0.71	0.00	0.00
	10	1.50	1.29	1.67	1.50	1.50	1.29	1.50	0.71	0.00	0.00
	12	2.33	3.00	1.67	1.71	1.71	1.29	1.50	0.71	0.00	0.00
	14	2.50	2.50	1.875	1.71	2.00	1.29	1.71	0.71	0.00	0.00
	16	2.875	2.20	2.33	2.29	1.875	1.00	1.71	0.71	0.00	0.00
	18	2.875	2.29	2.33	2.09	2.33	1.29	1.50	0.71	0.00	0.00
	20	1.67	4.00	1.875	2.00	1.50	1.50	1.33	0.71	0.00	0.00
	22	2.71	3.29	1.50	2.375	1.71	2.29	2.00	0.71	0.00	0.00
	24	2.50	2.80	1.67	1.50	2.00	1.50	2.00	0.00	0.00	0.00
	25	3.33	3.21	1.71	2.71	2.71	1.71	3.33	2.20	0.00	0.00
	25½	4.50	4.80	1.00	1.00	3.50	3.50	3.86	3.00	0.00	0.00
	26½	4.375	5.00	1.71	0.00	3.875	4.00	4.375	3.20	0.00	0.00
	27½	4.71	4.20	1.33	0.00	4.14	3.50	4.375	2.71	0.00	0.00
	28½	4.00	3.50	1.33	0.00	4.14	3.50	3.375	2.71	0.00	0.00
	29½	3.375	3.50	1.33	1.22	2.71	3.00	3.50	3.50	0.00	0.00
	30½	-	-	-	-	-	-	3.00	2.80	-	-
	32½	3.14	3.00	1.125	0.71	3.00	2.50	-	-	0.00	0.00
	34½	2.86	3.00	1.29	0.71	2.86	2.50	-	-	0.00	0.00
	36	3.67	2.50	0.83	0.71	3.00	2.29	-	-	0.00	0.00
	39	3.71	2.29	1.71	0.71	2.71	2.00	-	-	0.00	0.00
	41	3.33	2.71	1.67	0.71	3.125	2.00	-	-	0.00	0.00

Appendix I

Table 1.b Antibody analysis of serum ex Sow 88, taken 3½ weeks after farrowing to boar BFM2, i.e. at Week 27½.

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex W3184	Serum Absorbed Red Cells ex 72	Serum Absorbed Red Cells ex T38P12	Serum Absorbed Red Cells ex P20	SC
W3184	Aa Ea	V	-	V	++	V	-
72	Aa -	++	-	-	++	++	-
T38P12	Ao Ea	V	-	V	-	V	-
P20	Ao -	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex W3184	Serum Absorbed Red Cells ex 72	Serum Absorbed Red Cells ex T38P12	Serum Absorbed Red Cells ex P20	SC
W3184	Aa Ea	++	-	++	++	++	-
72	Aa -	++	-	-	++	++	-
T38P12	Ao Ea	++	-	++	-	++	-
P20	Ao -	-	-	-	-	-	-

Table 1.c Red Cell Typing of litter ex Sow 88 by Balhary FM2.

Animal No.		Red Cell Systems and Factors: A		D	E						F	G		I	K			L		Gb/Sc
		a	o	a	a	b	d	e	f	g	a	a	b	b	a	b	d	a	g	16
Sow	88	-	+	-	-	+	+	-	-	+	-	+	+	+	-	-	-	-	+	-
Boar	BFM2	-	+	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-
	56	-	-	-	+	+	+	-	-	+	-	-	+	-	-	+	-	-	+	-
	57	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-
	58	-	-	-	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+	-
	59	-	-	-	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+	-
	60	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	-	-	+	-
	61	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-
	62	-	-	-	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+	-
	63	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-
	64	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	+	+	-
	65	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-
	S.B.	-	-	-	-	+	+	-	-	+	-	-	+	+	-	+	-	-	+	-
	S.B.	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-

Appendix I

Table 2.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of animals in serial serum samples from Sow 405 during pregnancy and after parturition when mated to (1) an Ea negative boar (parturition at week 0) followed by (2) an Ea positive boar, BPM2 (parturition at Week 23).

Test Cell Donor		W3184		71P16		T38P12		P20	
Relevant Factors (Antigens)		Aa Ea		Aa -		Ao Ea		Ao -	
Serological Test		DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-4	2.33	4.50	2.33	4.29	1.50	0.71	0.00	0.00
	-2	2.50	4.29	2.33	4.29	1.50	0.71	0.00	0.00
	0	2.875	4.29	2.33	4.29	1.29	0.71	0.00	0.00
	2	2.875	3.71	2.125	3.71	1.29	0.71	0.00	0.00
	4	2.875	4.00	2.125	4.00	1.29	0.71	0.00	0.00
	6	3.33	4.00	2.33	4.00	1.50	0.71	0.00	0.00
	8	2.875	4.00	2.33	4.00	1.67	0.71	0.00	0.00
	10	2.00	5.00	2.50	3.00	1.50	0.00	0.00	0.00
	12	2.50	5.00	1.875	3.00	1.50	0.00	0.00	0.00
	14	1.71	3.00	1.50	3.00	1.50	0.00	0.00	0.00
	16	2.00	3.50	1.50	3.50	1.50	0.00	0.00	0.00
	18	2.125	4.00	1.71	4.00	1.50	0.00	0.00	0.00
	20	2.875	2.71	2.125	2.71	1.50	0.00	0.00	0.00
	22	2.875	3.00	2.33	2.71	1.50	0.71	0.00	0.00
	23	2.875	1.71	1.875	2.29	1.50	0.71	0.00	0.00
	23½	2.67	3.00	2.33	2.50	1.875	1.29	0.00	0.00
	24	3.125	3.00	2.33	2.50	2.33	2.50	0.00	0.00
	24½	3.86	3.71	2.125	3.00	2.875	2.71	0.00	0.00
	26	2.71	5.50	2.00	3.80	2.00	1.50	0.00	0.00
	27	2.33	4.50	1.875	4.00	1.71	1.71	0.00	0.00
	28	2.125	3.50	1.80	3.00	2.00	1.50	0.00	0.00
	30	1.86	3.50	1.50	2.50	1.50	1.00	0.00	0.00
	31	1.33	2.00	0.67	2.00	1.33	1.00	0.00	0.00

Table 2.b Antibody analysis of serum ex Sow 405, taken 1½ weeks after farrowing to boar BPM2, i.e. Week 24½.

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex.				SC
			W3184	72	T38P12	P20	
W3184	Aa Ea	V	-	V	V	V	-
72	Aa -	V	-	-	++	V	-
T38P12	Ao Ea	V	-	++	-	++	-
P20	Ao -	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex.				SC
			W3184	72	T38P12	P20	
W3184	Aa Ea	++	-	++	++	++	-
72	Aa -	++	-	-	++	++	-
T38P12	Ao Ea	++	-	++	-	++	-
P20	Ao -	-	-	-	-	-	-

Table 2.c Red Cell Typing of litter ex Sow 405 by BPM2:

DA 1.5% 1½h 37°C Micro

Antigen	Reagent	FD	Piglet No.													
			29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Ea	71	1/5	+	++	-	V	++	V	++	V	++	++	-	-	-	-	++
Ea	B3-1554	1/5	(+)	++	-	++	++	++	++	++	V	V	-	-	-	-	++

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Table 3.a $\log_2$ of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of five animals in serial serum samples from 301 during pregnancy and after parturition to BFM2 (Week 0).

Test Cell Donor	W3184		305		T38P12		RW		L413	
Relevant Factors (Antigens)	Aa Ea Kb		Aa - -		Ao Ea Kb		Ao - Kb		Ao - -	
Serological Techniques	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
-20	2.50	10.50	1.875	7.00	1.33	0.00	1.125	0.00	0.00	0.00
-17	2.71	9.29	1.86	9.14	1.125	0.00	0.67	0.00	0.00	0.00
-14	2.50	7.29	2.20	6.29	1.125	0.00	0.67	0.00	0.00	0.00
-10	2.71	8.20	1.50	7.71	0.875	0.00	1.125	0.00	0.00	0.00
-8	3.71	10.50	2.50	8.50	3.00	3.71	2.22	0.00	0.00	0.00
-6	6.67	9.71	2.33	7.80	6.14	7.20	0.875	0.00	0.00	0.00
-3	7.43	9.50	3.00	8.29	6.67	7.00	2.33	1.50	0.00	0.00
-1	6.17	9.71	3.14	7.20	4.59	7.00	1.125	2.50	0.00	0.00
-½	5.80	9.71	3.20	7.71	4.71	6.71	1.125	1.50	0.00	0.00
0	4.33	10.50	1.86	7.00	3.25	5.00	1.50	1.29	0.00	0.00
½	5.00	9.00	2.29	7.50	4.00	5.71	1.50	1.00	0.00	0.00
1	4.71	8.29	3.29	7.50	4.14	6.29	1.25	1.80	0.00	0.00
1½	4.71	8.00	3.125	6.50	4.29	5.50	1.33	2.50	0.00	0.00
2	5.50	7.20	2.50	7.00	4.71	5.71	1.50	2.50	0.00	0.00
3	4.50	7.71	2.125	7.50	3.71	6.00	0.875	2.00	0.00	0.00
4	3.33	7.71	1.50	6.71	3.125	5.71	1.125	1.00	0.00	0.00
6	3.14	7.00	1.50	7.00	3.67	5.50	1.125	1.50	0.00	0.00
8	4.67	7.29	2.67	7.00	3.375	5.50	2.125	2.00	0.00	0.00
10	3.50	9.50	1.20	9.50	3.43	5.29	1.71	1.29	0.00	0.00
12	2.50	10.29	1.67	9.71	2.57	4.50	1.33	0.71	0.00	0.00

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Table 3.b Antibody analysis of serum ex 301 (23.7.68).

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	Unabsorbed Serum 1:4	Serum Absorbed Red Cells ex W3184 1:2	W3184 1:4	305 1:2	305 1:4	T38P12 1:2	T38P12 1:4	RW 1:2	RW 1:4	L413 1:2	L413 1:4	SC
W3184	Aa Ea Kb	V	V	-	-	V	V	++	++	V	V	V	V	-
305	Aa - -	++	+(+)	-	-	-	-	++	+	++	(+)	++	+	-
T38P12	Ao Ea Kb	V	V	-	-	V	V	-	-	V	V	V	V	-
RW	Ao - Kb	++	-	-	-	+(+)	-	-	-	-	-	+(+)	-	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	Unabsorbed Serum 1:4	Serum Absorbed Red Cells ex W3184 1:2	W3184 1:4	305 1:2	305 1:4	T38P12 1:2	T38P12 1:4	RW 1:2	RW 1:4	L413 1:2	L413 1:4	SC
W3184	Aa Ea Kb	++	++	-	-	++	++	++	++	++	++	++	++	-
305	Aa - -	++	++	-	-	-	-	++	++	++	++	++	++	-
T38P12	Ao Ea Kb	++	++	-	-	++	++	-	-	++	++	++	++	-
RW	Ao - Kb	++	-	-	-	+	-	-	-	-	-	+	-	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.c Red cell typing of litter ex 301 by BFM2.

Animal No.	Red Cell Systems and Factors: A a	A o	D a	E a	E b	E d	E e	E f	E g	F a	F b	G a	G b	I b	K a	K b	K d	L a	L g	Gb.Sc. 9	Gb.Sc. 16
Sow 301	-	+	-	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	+	-	-
Boar BFM2	-	+	-	+	+	+	+	-	+	-	+	-	+	+	-	+	-	-	+	+	-
321	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	+	-	-	+	+	-
322	-	-	-	-	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	-
323	-	-	-	+	+	+	+	-	+	-	+	-	+	+	-	+	-	+	+	+	-
324	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	+	-	-	+	+	-
325	-	-	-	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	+	+	-
326	-	-	-	+	+	+	+	-	+	-	+	-	+	+	-	+	-	+	+	+	-
327	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	+	-	+	+	+	-
328	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-
329	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-
330	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-
331	-	-	-	+	+	+	+	-	+	-	+	-	+	-	+	+	+	-	+	+	-
332	-	-	-	+	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+	+	-

Appendix I

Table 4.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of animals in serial serum samples from Sow 301 during pregnancy and after parturition (Week 0) to boar 8.

Test Cell Donor		8544		311		8		RW		191		L413	
Relevant Factors (Antigens)		Aa - Kb		Aa - Kb		Ao Ea Kb		Ao - Kb		Ao - Kb		Ao - -	
Serological Tests		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-11	2.71	9.29	2.33	9.29	2.67	4.00	1.71	0.00	1.71	1.29	0.00	0.00
	-9	3.375	8.29	2.57	6.80	3.33	4.29	2.00	0.00	2.14	2.29	0.00	0.00
	-7	3.83	8.50	3.25	8.20	3.43	5.50	3.67	5.50	3.67	5.29	0.00	0.00
	-4	5.71	9.00	4.83	8.00	5.71	7.50	5.14	7.71	5.14	7.29	0.00	0.00
	-1½	4.60	7.14	4.17	7.50	4.29	6.50	4.00	5.50	4.17	6.29	0.00	0.00
	-½	3.29	6.14	2.50	7.50	3.43	5.50	4.00	6.14	3.17	4.71	0.00	0.00
	½	3.25	7.50	3.375	7.50	4.22	6.50	3.00	6.50	3.80	6.50	0.00	0.00
	1½	5.00	7.29	3.57	7.29	2.57	6.29	3.17	6.29	4.29	7.375	0.00	0.00
	3	3.40	7.50	4.22	7.71	4.11	6.29	4.33	5.50	4.22	6.50	0.00	0.00

Table 4.b Antibody analysis of serum ex 301, taken four weeks before parturition (Week -4).

Direct Agglutination Test

Cell Donor	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex										*SC
				8544		311		8		RW		L413		
		1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	
8544	Aa Ea Kb	V	V	-	-	++	++	++	++	V	V	V	V	-
311	Aa - Kb	++	++	-	-	-	-	++	++	++	++	++	+(+)	-
8	Ao Ea Kb	V	V	-	-	++	++	-	-	++	++	V	V	-
RW	Ao - Kb	++	++	-	-	-	-	-	-	-	-	++	+(+)	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Donor	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex										SC
				8544		311		8		RW		L413		
		1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	
8544	Aa Ea Kb	++	++	-	-	++	++	++	++	++	++	++	++	-
311	Aa - Kb	++	++	-	-	-	-	++	++	++	++	++	++	-
8	Ao Ea Kb	++	++	-	-	++	++	-	-	++	++	++	++	-
RW	Ao - Kb	++	++	-	-	-	-	-	-	-	-	++	++	-
L413	Ao - -	++	++	-	-	-	-	-	-	-	-	-	-	-

Table 4.c Red Cell Typing of Litter ex 301 by 8

DA 37°C 2% 1½ hrs. Micro

Antigen	Reagent	FD	Animal No.										
			384	385	386	387	388	389	390	391	392	301	8
Ea	71	1/5	-	-	-	+(+)	-	++	-	-	++	-	++
Saline Control			-	-	-	-	-	-	-	-	-	-	-

IST 2.0% 1° 37°C. 2% 1 h. 37°C. AFG R5 1/50 hrs.

Antigen	Reagent	FD	Animal No.										
			384	385	386	387	388	389	390	391	392	301	8
Kb	75	1/30	++	-	-	++	++	-	-	++	-	-	++
Saline Control			-	-	-	-	-	-	-	-	-	-	-

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Table 5.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the relevant antigens present on the red cells of a panel of animals in serial serum samples from Sow 301 during pregnancy and after parturition (Week 0) to 312.

Test Cell Donor		8544		311		8		RW		191		L413	
Relevant Factors (Antigens)		Aa Ea Kb		Aa - Kb		Ao Ea Kb		Ao - Kb		Ao - Kb		Ao - -	
Serological Tests		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-13	3.17	6.50	3.17	6.50	3.40	5.50	3.83	3.29	2.83	5.29	0.00	0.00
	-11	3.17	7.50	3.17	7.50	2.83	5.50	2.57	4.00	3.22	4.50	0.00	0.00
	-9	3.17	7.29	2.57	7.50	3.11	7.14	3.875	5.80	3.00	5.00	0.00	0.00
	-7	3.17	7.50	3.40	7.50	2.86	6.00	3.11	3.50	3.29	5.00	0.00	0.00
	-5	3.75	6.71	3.33	7.29	3.14	5.00	2.40	3.71	2.00	4.50	0.00	0.00
	-3	3.50	7.29	3.71	7.14	3.33	6.29	2.71	4.00	2.83	4.50	0.00	0.00
	-2	2.83	7.375	3.50	7.50	3.17	6.00	2.83	5.29	3.00	5.29	0.00	0.00
	-1	2.83	6.29	2.57	5.50	2.50	6.14	2.14	5.29	1.71	4.29	0.00	0.00
	-½	1.50	6.29	2.83	6.50	2.33	4.50	2.57	2.71	1.125	4.00	0.00	0.00
	½	2.125	7.29	1.71	6.50	1.50	3.71	1.50	1.00	1.29	4.29	0.00	0.00
	⅔	2.17	7.50	1.67	6.29	1.50	5.00	1.125	3.20	2.00	4.29	0.00	0.00
	1½	2.71	8.00	2.86	6.50	2.83	5.50	1.83	4.29	2.33	4.00	0.00	0.00
	2½	3.00	7.29	2.33	7.00	2.33	4.71	1.80	4.00	2.50	4.50	0.00	0.00
	3½	3.00	6.50	2.57	5.71	2.14	5.50	1.71	3.29	2.33	4.00	0.00	0.00
	4½	2.125	6.50	2.125	6.00	2.22	4.71	1.71	3.29	1.50	3.50	0.00	0.00

Table 5.b Red cell typing of litter ex 301 by 312.

DA 37°C 1.5% 1½ h. Micro

Antigen	Reagent	FD	Piglet No.				
			461	462	463	464	465
Ea	B3/1554	1/5	-	-	-	-	-
Ea	71	1/5	-	-	-	-	-
Saline Control			-	-	-	-	-

IST 2.0% 1½ h. 37°C. 2% 1 h. 37°C. APG R5 1/50 Macro

Antigen	Reagent	FD	Piglet No.				
			461	462	463	464	465
Kb	75	1/30	-	-	-	-	-
Saline control			-	-	-	-	-

Appendix I

Table 6.a Log$_2$ of the reciprocals of the 50% end point titres of the antibodies to the relevant antigens present on the red cells of a panel of animals in serial serum samples from sow 302 during the latter stages of pregnancy and after parturition (Week 0) to a boar which was Ea positive, Kb positive.

Test Cell Donor		W3184		PY4731		T38P12		RW		L413	
Relevant Factors (Antigens)		Aa Ea Kb		Aa - Kb		Ao Ea Kb		Ao - Kb		Ao - -	
Serological Tests		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
	-1½	4.33	8.00	3.71	8.00	1.33	0.00	0.00	0.00	0.00	0.00
	2	6.57	8.50	3.00	8.25	6.25	7.80	2.71	3.50	0.00	0.00
TIME	4	7.00	10.50	3.50	6.80	6.20	5.00	-	-	0.00	0.00
(WEEKS)	7	5.80	8.80	1.77	8.20	4.71	4.80	1.33	0.00	0.00	0.00
	9	5.14	6.50	3.00	6.50	5.33	3.50	1.33	0.00	0.00	0.00

Table 6.b Antibody analysis of serum ex 302 taken 2 weeks post partum (Week 2).

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum absorbed with Red Cells ex: W3184		PY4731		T38P12		P20		RW		L413		SC
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa Ea Kb	V	V	-	-	V	V	++	+	V	V	V	V	V	V	-
PY4731	Aa - Kb	++	+(+)	-	-	-	-	++	(+)	++	(+)	++	(+)	++	(+)	-
T38P12	Ao Ea Kb	V	V	-	-	V	V	-	-	V	V	V	V	V	V	-
P20	Ao - Kb	++	+	-	-	-	-	-	-	-	-	-	-	++	(+)	-
RW	Ao - Kb	++	+	-	-	-	-	-	-	-	-	-	-	++	(+)	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum absorbed with Red Cells ex: W3184		PY4731		T38P12		P20		RW		L413		SC
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa Ea Kb	++	++	-	-	++	++	++	++	++	++	++	++	++	++	-
PY4731	Aa - Kb	++	++	-	-	-	-	++	++	++	++	++	++	++	++	-
T38P12	Ao Ea Kb	++	++	-	-	++	++	-	-	++	++	++	++	++	++	-
P20	Ao - Kb	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-
RW	Ao - Kb	++	+	-	-	-	-	-	-	-	-	-	-	-	-	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6.c Red cell typing of litter ex sow 302 by an Ea positive, Kb positive boar.

Animal No.	Red Cell Systems and Factors: A		D	E						F	G		I	K			L		Gb/Sc
	a	o	a	a	b	d	e	f	g	a	a	b	b	a	b	d	a	g	16
Sow 302	-	+	-	-	+	+	+	-	+	-	+	+	+	+	-	+	-	+	-
Boar	-	+	+	+	-	+	+	-	+	-	+	+	-	+	+	+	-	+	-
12	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+	-
13	-	+	-	-	-	+	+	-	+	-	-	+	+	+	+	+	-	+	-
14	-	+	-	+	+	+	+	-	+	-	+	+	+	-	+	-	-	+	-
15	-	+	-	+	-	+	+	-	+	-	+	+	+	-	+	-	-	+	-
16	-	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+	-	+	-

Appendix I

Table 7.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the relevant antigens present on the red cells of a panel of animals in serial serum samples from Sow 302 during pregnancy and after parturition (Week 0) to BFM2.

Test Cell Donor		W3184		305		T38P12		RW		L413	
Relevant Factors (Antigens)		Aa Ea Kb		Aa - -		Ao Ea Kb		Ao - Kb		Ao - -	
Serological Tests		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-16	5.80	8.80	1.77	8.20	4.71	4.80	1.33	0.00	0.00	0.00
	-14	5.14	6.50	3.00	6.50	5.33	3.50	1.33	0.00	0.00	0.00
	-10	4.86	10.00	3.125	8.80	4.50	3.00	1.33	0.00	0.00	0.00
	-8	5.14	8.50	2.50	8.29	4.14	3.50	1.33	0.00	0.00	0.00
	-6	4.00	6.71	3.71	6.71	4.50	3.71	1.50	0.00	0.00	0.00
	-3	5.33	7.29	4.00	6.71	4.50	4.29	1.125	0.00	0.00	0.00
	-1	5.17	7.00	3.50	6.50	3.50	4.71	1.125	0.00	0.00	0.00
	0	7.67	9.00	3.29	7.29	7.00	8.50	2.50	4.50	0.00	0.00
	½	7.33	10.00	3.33	7.50	7.17	10.00	3.33	5.00	0.00	0.00
	¾	8.00	10.50	2.40	7.50	8.00	9.80	3.20	6.00	0.00	0.00
	1½	8.00	10.00	2.40	6.50	8.25	9.00	4.125	4.29	0.00	0.00
	2	10.60	10.71	2.40	7.00	11.125	10.71	5.40	6.29	0.00	0.00
	3	9.40	9.50	2.40	6.29	8.17	9.00	5.00	5.50	0.00	0.00
	4	7.50	8.50	2.14	6.50	7.71	8.50	3.33	4.29	0.00	0.00
	6	3.00	8.50	2.86	6.00	7.33	8.80	2.67	4.00	0.00	0.00
	8	6.33	7.50	2.875	7.00	6.86	7.00	2.33	3.29	0.00	0.00
	10	7.00	9.50	2.14	8.71	6.67	7.29	1.60	2.29	0.00	0.00

Appendix I

Table 7.b Antibody analysis of serum ex Sow 302 taken 1½ weeks after parturition to boar, BFM2 (Week 1½).

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex										SC
				W3184		305		T38P12		RW		L413		
		1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	
W3184	Aa Ea Kb	V	V	-	-	V	V	++	+	V	V	V	V	-
305	Aa - -	V	+	-	-	-	-	++	(+)	++	(+)	++	+	-
T38P12	Ao Ea Kb	V	V	-	-	V	V	-	-	V	V	V	V	-
RW	Ao - Kb	V	+(+)	-	-	++	+	-	-	-	-	V	+(+)	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Indirect Sensitisation Test

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex										SC
				W3184		305		T38P12		RW		L413		
		1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	1:4	1:8	
W3184	Aa Ea Kb	++	++	-	-	++	++	++	++	++	++	++	++	-
305	Aa - -	++	++	-	-	-	-	++	++	++	++	++	++	-
T38P12	Ao Ea Kb	++	++	-	-	++	++	-	-	++	++	++	++	-
RW	Ao - Kb	++	+(+)	-	-	+(+)	+	-	-	-	-	++	+	-
L413	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 7.c Red Cell Typing of litter ex Sow 302 by BFM2.

Animal No.		Red Cell Systems and Factors																													
		A		D	E						F		G		I	K			L		Gb.Sc.										
		a	o	a	a	b	d	e	f	g	a	b	a	b	b	a	b	d	a	g	9	16									
Sow	302	-	+	-	-	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	+	-									
	BFM2	-	+	-	+	+	+	+	-	+	-	+	-	+	+	-	+	-	-	+	+	-									
	333	-	-	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-									
	334	-	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	335	-	-	-	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	336	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-	-	+	+	-									
	337	-	-	-	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	-									
	338	-	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	339	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	340	-	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	341	-	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-									
	342	-	-	-	+	+	+	+	-	+	-	+	-	+	-	-	+	-	-	+	+	-									

Appendix I

Table 8.a Log_2 of the reciprocals of the 50% end point titres of antibodies to the relevant antigens present in the red cells of a panel of animals in serial serum samples from sow 302 during pregnancy and after parturition (Week 0) to 311.

Test Cell Donor		8544		311		8		RW		L412		191	
Relevant Factors (Antigens)		Aa Ea - Kb		Aa - Ef Kb		Ao Ea - Kb		Ao - - Kb		Ao - Ef -		Ao - - Kb	
Serological Tests		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-16	5.14	6.71	3.57	6.50	4.14	4.71	1.125	0.71	0.875	0.00	2.00	0.71
	-12	4.50	6.50	3.43	6.50	4.50	5.50	1.71	2.25	0.875	0.00	1.71	3.00
	-9	4.57	6.00	2.71	5.50	3.83	6.00	1.71	1.00	0.00	0.00	1.71	2.50
	-7	4.29	6.50	2.33	6.50	4.29	5.50	1.71	1.00	0.00	0.00	2.00	1.50
	-4	4.00	7.00	2.60	6.50	4.17	6.29	2.14	2.50	0.875	0.00	2.14	2.50
	-2½	4.83	7.00	2.57	5.50	5.14	5.50	1.71	2.29	0.875	0.00	2.33	2.29
	-1½	4.29	6.00	2.33	4.71	4.17	7.00	2.00	1.71	0.875	0.00	1.71	1.71
	0	3.83	7.00	1.71	6.50	3.875	6.20	2.83	1.50	0.875	0.00	2.33	3.00
	½	6.00	10.80	5.86	10.50	5.33	6.00	2.83	4.29	1.125	2.71	1.71	3.50
	1½	5.00	8.50	4.33	8.29	4.86	5.71	1.71	4.00	1.33	3.71	1.71	4.00
	2½	4.43	8.50	1.67	8.50	3.33	5.29	1.125	3.50	0.00	2.50	0.875	2.50
	3½	4.67	9.14	3.57	8.29	4.57	5.29	1.33	3.00	0.67	2.29	1.125	2.29
	4½	6.00	8.50	3.49	7.29	4.17	5.71	1.71	2.71	1.71	2.50	2.00	2.71
	5½	4.00	7.50	3.00	6.50	4.00	5.00	1.33	2.50	0.67	1.50	1.33	2.00
	6½	4.29	7.29	2.50	7.00	4.29	5.29	1.71	2.00	0.875	1.29	1.125	2.00

Table 8.b Antibody analysis of serum ex 302 taken 1½ weeks post partum (Week 1½).

Direct Agglutination Test

Cell Donor	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex														SC
				8544		311		8		RW		L412		191				
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4			
8544	Aa Ea - Kb	V	V	-	-	V	V	++	+(+)	V	V	V	V	V	V	-		
311	Aa - Ef Kb	V	++	+	-	-	-	++	++	++	++	++	+	++	++	-		
8	Ao Ea - Kb	V	V	-	-	V	V	-	-	V	V	V	V	V	V	-		
RW	Ao - - -	++	+	-	-	-	-	-	-	-	-	++	(+)	-	-	-		
L412	Ao - Ef -	++	-	+(+)	-	-	-	+	-	+	-	-	-	+	-	-		
191	Ao - - Kb	++	+	-	-	-	-	-	-	-	-	+(+)	+	-	-	-		

Indirect Sensitisation Test

Cell Donor	Relevant Antigens	Unabsorbed Serum		Serum Absorbed with Red Cells ex														SC
				8544		311		8		RW		L412		191				
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4			
8544	Aa Ea - Kb	++	++	-	-	++	++	++	++	++	++	++	++	++	++	-		
311	Aa - Ef Kb	++	++	++	+	-	-	++	++	++	++	++	++	++	++	-		
8	Ao Ea - Kb	++	++	-	-	++	++	-	-	++	++	++	++	++	++	-		
RW	Ao - - Kb	++	++	-	-	-	-	-	-	-	-	++	+(+)	-	-	-		
L412	Ao - Ef -	++	++	++	(+)	-	-	++	+	++	(+)	-	-	++	(+)	-		
191	Ao - - Kb	++	++	-	-	-	-	-	-	-	-	++	+	-	-	-		

Table 8.c Red cell typing of litter ex Sow 302 by 311.

DA 37°C. 1.5% 1½ h. Micro

Antigen	Reagent	FD	Piglet No.										
			421	422	423	424	425	426	427	428	429	430	431
Ef	78	1/6	++	-	-	-	V	++	-	-	-	++	-
Saline Control			-	-	-	-	-	-	-	-	-	-	-

IST 2% 1½ h. 37°C. 2% 1 h. 37°C. APG R5 1/50 Macro.

Antigen	Reagent	FD	Piglet No.										
			421	422	423	424	425	426	427	428	429	430	431
Kb	75	1/30	++	++	++	-	-	++	-	-	-	-	-
La	68	1/8	++	++	-	-	++	-	++	-	++	++	++
Saline Control			-	-	-	-	-	-	-	-	-	-	-

Appendix I

Table 9.a Log_2 of the reciprocals of the 50% end point titres of anti-Ea in the sera of gilts 1347 and 1350 and sow 1113 as detected by reactions with red cells from boar 8.

Test Cell Donor		1347		1350		1113	
Serological Tests		DA	IST	DA	IST	DA	IST
TIME (WEEKS)	-4	0.00	0.00	0.00	0.00	0.00	0.00
	-2	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00
	1	0.67	0.00	1.125	0.00	1.71	0.00
	2	0.67	0.00	1.125	0.00	3.375	0.00
	3	0.00	0.00	1.125	0.00	3.375	0.00
	4	0.00	0.00	1.125	0.00	2.22	0.00

Table 9.b Serum ex 1350 at Week 2.

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex				SC
			8544	311	8	RW	
8544	Aa Ea	V	-	++	++	V	-
311	Aa -	++	-	-	++	++	-
8	Ao Ea	V	-	++	-	V	-
RW	Ao -	-	-	-	-	-	-

Table 9.c Serum ex 1113 at week 2.

Direct Agglutination Test

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex				SC
			8544	311	8	RW	
8544	Aa Ea	V	-	V	V	V	-
311	Aa -	V	-	-	++	++	-
8	Ao Ea	V	-	V	-	V	-
RW	Ao -	-	-	-	-	-	-

APPENDIX II

Appendix II

Table 1.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of five animals in serum samples taken from adult boars.

Boar No.	Donor of Test Red Cells									
	140		8595		12P22		306		191	
	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
8983	1.00	5.125	2.00	9.20	0.00	0.00	0.00	0.00	0.00	0.00
7054	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2592	0.67	1.50	0.875	4.50	0.00	0.00	0.00	0.00	0.00	0.00
267	0.67	0.00	1.33	4.80	0.00	0.00	0.00	0.00	0.00	0.00
4684	0.00	5.50	1.125	9.00	0.00	0.00	0.00	0.00	0.00	0.00
3430	0.00	2.29	0.00	7.50	0.00	0.00	0.00	0.00	0.00	0.00
7446	0.00	5.00	1.50	7.50	0.00	0.00	0.00	0.00	0.00	0.00
4628	1.50	1.50	2.33	5.00	0.00	0.00	0.00	0.00	0.00	0.00
7919	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3611	0.00	5.50	0.67	10.29	0.00	0.00	0.00	0.00	0.00	0.00
5355	1.125	1.50	2.86	6.67	0.00	0.00	0.00	0.00	0.00	0.00
8739	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7660	1.125	3.50	2.71	10.00	0.00	0.00	0.00	0.00	0.00	0.00
2224	0.67	3.80	2.22	5.80	0.00	0.00	0.00	0.00	0.00	0.00
7722	0.875	5.00	1.125	8.00	0.00	0.00	0.00	0.00	0.00	0.00
7950	0.00	6.50	1.33	10.50	0.00	0.00	0.00	0.00	0.00	0.00
5448	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7447	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8439	0.875	3.50	1.50	8.14	0.00	0.00	0.00	0.00	0.00	0.00

Table 1.b

Boar No.	Donor of Test Red Cells									
	311		308		BWR		L412		191	
	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
1381	2.83	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8739	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
651	0.00	0.00	0.00	0.00	0.67	0.00	0.67	0.00	0.875	0.00
6527	1.67	-	0.00	-	0.00	-	0.00	-	0.00	-
3912	2.29	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.875	0.00
4692	2.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00
6029	1.29	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6266	1.50	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8368	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5918	1.125	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8729	2.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9465	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9535	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3776	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2843	1.71	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6390	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.875	0.00
3197	1.20	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3422	3.49	5.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6010	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4283	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7547	1.50	3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1847	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8742	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6328	1.50	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00
3667	1.50	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2898	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3273	2.33	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8103	3.17	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4097	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2652	3.33	5.50	0.67	0.00	0.67	0.00	0.00	0.00	0.00	0.00
2067	1.125	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6413	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2423	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1692	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5134	2.22	2.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6867	2.22	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4439	3.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2169	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1895	1.50	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1021	2.57	5.50	0.00	0.00	0.875	0.00	0.00	0.00	0.00	0.00
12875	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2743	1.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6621	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4147	1.66	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1526	2.17	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1919	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6698	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5883	1.125	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix II

Table 2.a Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present in the red cells of a panel of five animals in serum samples taken from adult sows.

Sow No.	Breed	Donor of Test Red Cells									
		140		8595		12P42		308		191	
		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
7335	LR	0.875	3.00	2.00	5.80	0.00	0.00	0.00	0.00	0.00	0.00
7734	LR	0.00	0.00	0.67	0.00	0.00	0.00	0.67	0.00	0.00	0.00
8328	LR	0.875	7.00	1.125	10.00	0.67	0.00	0.00	0.00	0.00	0.00
2523	LW	0.875	4.80	1.50	9.71	0.00	0.00	0.00	0.00	0.00	0.00
2567	LW	1.875	3.29	3.50	9.00	0.00	0.00	0.00	0.00	0.00	0.00
8193	LR	0.875	3.50	1.50	6.71	0.00	0.00	0.00	0.00	0.00	0.00
8194	LR	0.875	1.83	3.00	7.29	0.00	0.00	0.00	0.00	0.00	0.00
1486	LR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2123	LW	0.875	0.00	0.00	0.00	0.875	0.00	0.00	0.00	0.00	3.50
10325	W	2.14	1.29	3.00	9.50	0.00	0.00	0.00	0.00	0.00	0.00
2793	LW	0.00	4.20	1.71	9.50	0.00	0.00	0.00	0.00	0.00	0.00
3649	W	0.875	5.50	1.71	9.50	0.00	0.00	0.875	0.00	0.67	0.00
8340	LR	1.125	0.00	1.29	5.29	0.00	0.00	0.67	0.00	0.00	0.00
564	LR	0.875	0.00	0.00	2.29	1.29	0.00	0.00	0.00	0.00	0.00
9035	LR	0.67	3.00	1.71	7.00	0.00	0.00	0.00	0.00	0.00	0.00
8117	W	0.67	5.00	2.14	9.50	0.00	0.00	0.00	0.00	0.00	0.00
7229	LW	0.00	0.00	3.00	3.50	3.00	3.00	3.00	4.17	0.00	0.00
5505	LR	0.875	3.50	3.375	8.71	0.00	0.00	0.00	0.00	0.00	0.00
4505	LR	0.67	2.29	1.33	7.80	0.00	0.00	0.00	0.00	0.00	0.00
8483	LW	1.29	5.00	2.71	5.00	0.00	0.00	0.00	0.00	0.00	0.00
5806	LR	1.50	1.00	2.125	5.71	0.00	0.00	0.00	0.00	0.00	0.00
3733	LR	1.33	0.00	1.33	0.00	1.125	0.00	1.50	0.00	1.33	0.00
6276	LR	1.33	4.80	2.71	9.50	1.125	0.00	1.33	0.00	1.35	0.00
3015	LW	0.875	3.50	1.33	5.00	0.00	0.00	0.00	0.00	0.00	0.00
1416	LW	0.00	3.83	1.71	7.80	0.00	2.50	1.50	0.00	0.67	1.00
2378	LR	0.67	0.00	1.00	6.00	0.875	6.50	0.67	5.29	0.00	0.00
1993	LW	0.67	4.29	0.67	8.50	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.b

Sow No.	Breed	Donor of Test Red Cells									
		311		308		BWR		L412		191	
		DA	IST	DA	IST	DA	IST	DA	IST	DA	IST
6420	LR	0.00	-	0.00	-	3.00	-	0.00	-	0.00	-
3866	LR	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
106	LW	1.71	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
341	SB	1.71	4.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3907	W	2.00	5.50	0.00	0.00	1.125	0.00	0.00	0.00	1.125	0.00
4219	LR	1.125	0.00	3.00	3.71	0.00	0.00	2.14	1.00	2.00	1.38
4936	LR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3537	LW	1.80	5.50	3.71	5.50	0.00	0.00	0.00	0.00	2.33	4.50
7526	LW	0.00	-	0.00	-	0.67	-	0.00	-	0.67	-
9142	LR	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4450	LR	0.00	5.50	0.00	0.00	1.125	0.00	0.00	0.00	0.00	0.00
4656	LR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303	LR	2.875	5.50	0.875	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9570	LR	2.14	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
185	LW	0.875	5.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3127	LW	1.86	4.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6232	LR	1.00	5.50	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3312	LW	0.00	0.00	0.00	0.00	1.71	1.29	0.00	0.00	0.00	0.00
3748	LW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0965	LW	1.40	5.50	2.33	2.29	0.00	0.00	0.00	0.00	0.00	0.00
1006	W	0.00	5.50	0.875	1.20	0.00	0.00	0.00	0.00	0.00	0.00
6656	LW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
308	LW	2.33	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3717	LW	2.57	5.50	0.875	0.00	0.00	0.00	0.00	0.00	0.875	0.00
5908	LR	1.71	5.50	0.00	0.00	1.71	2.29	0.00	0.00	0.00	0.00
5214	LR	0.00	0.00	0.00	0.00	1.125	0.00	0.00	0.00	0.875	0.00
5598	LR	1.71	5.50	1.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7430	LW	1.86	4.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3754	LR	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3904	W	2.33	5.50	1.00	1.00	0.00	0.00	0.875	0.00	1.33	0.00
2274	LW	3.17	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2135	LW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2346	W	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3330	LW	0.00	0.00	0.00	0.00	2.33	0.00	0.00	0.00	0.00	0.00
5670	LR	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2123	LW	3.00	5.50	0.00	0.00	1.50	0.00	0.00	0.00	0.00	0.00
1965	LW	1.71	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3536	LR	1.125	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4663	LR	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3855	LR	3.375	5.50	0.875	0.00	0.875	0.00	0.00	0.00	0.00	0.00
3433	LR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4887	LW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1109	LW	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13158	W	3.17	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3801	LW	2.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6784	LW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
687	LR	1.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1110	LW	3.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7530	LW	2.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8601	LR	1.71	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1209	LW	0.875	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9191	LW	2.14	3.50	0.67	0.00	0.00	0.00	0.00	0.00	0.67	0.00
335	W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

LW - Large White LR - Landrace W - Welsh SB - Saddleback

Appendix II

Table 3 Identification of iso-antibodies present in the sera of adult sows (Appendix II, Tables 2a and b) by checkerboard analysis.

Serum ex 2123

1ST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Serum Absorbed Red Cells ex 191	301	W3184	8595	SC
191	Eb Ed	++	-	-	++	++	-
301	- Ed	++	-	-	++	++	-
W3184	Eb -	-	-	-	-	-	-
8595	- -	-	-	-	-	-	-

Serum ex 564

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Serum Absorbed Red Cells ex 12P22	140	8595	191	306	SC
12P22	Ao Ea	+(+)	-	-	+(+)	+	+	-
140	Aa Ea	+	-	-	+	+	(+)	-
8595	Aa -	-	-	-	-	-	-	-
191	Ao -	-	-	-	-	-	-	-
306	Ao -	-	-	-	-	-	-	-

Serum ex 7229

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	1:2	Serum Absorbed Red Cells ex 306 1:1	1:2	8595 1:1	1:2	12P22 1:1	1:2	191 1:1	1:2	140 1:1	1:2	SC
306	Ao Eb	++	++	-	-	-	-	-	-	++	++	++	++	-
8595	Aa Eb	V	++	-	-	-	-	-	-	++	+(+)	++	+(+)	-
12P22	Ao Eb	V	++	-	-	-	-	-	-	++	+(+)	++	+(+)	-
191	Ao -	-	-	-	-	-	-	-	-	-	-	-	-	-
140	Aa -	-	-	-	-	-	-	-	-	-	-	-	-	-

1ST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	1:4	Serum Absorbed Red Cells ex 306 1:2	1:4	8595 1:2	1:4	12P22 1:2	1:4	191 1:2	1:4	140 1:2	1:4	SC
306	Ao Eb	++	++	-	-	-	-	-	-	++	+	++	(+)	-
8595	Aa Eb	++	+	-	-	-	-	-	-	++	(+)	++	(+)	-
12P22	Ao Eb	++	+	-	-	-	-	-	-	++	+	++	(+)	-
191	Ao -	-	-	-	-	-	-	-	-	-	-	-	-	-
140	Aa -	-	-	-	-	-	-	-	-	-	-	-	-	-

Serum ex 3733

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Serum Absorbed Red Cells ex 191	140	306	8595	12P22	L412	BWR	301	SC
191	Eo Eg Eb	++	-	-	-	-	-	++	-	+(+)	-
140	Eo Eg Eb	++	-	-	-	-	-	+(+)	-	+(+)	-
306	Eo Eg Eb	++	-	-	-	-	-	+(+)	-	++	-
8595	Eo Eg Eb	++	-	-	-	-	-	+(+)	-	+	-
12P22	Eo Eg Eb	++	-	-	-	-	-	+(+)	-	+	-
L412	Eo - -	-	-	-	-	-	-	-	-	-	-
BWR	- Eg Eb	++	-	-	-	-	-	+(+)	-	+(+)	-
301	- Eg -	-	-	-	-	-	-	-	-	-	-

L412 and 301 carry factors Gb; Eg; Lb; Lt; Kb.

Appendix II

Table 3 (Contd.)

Serum ex 6276

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex								SC
			191	140	306	8595	12P22	L412	BWR	301	
191	Ao Ee	++	-	-	-	-	-	-	+(+)	+(+)	-
140	Aa Ee	++	+	-	+	-	+	+	++	++	-
306	Ao Ee	++	-	-	-	-	-	-	+	+(+)	-
8595	Aa Ee	V	++	-	++	-	++	++	V	V	-
12P22	Ao Ee	+(+)	-	-	-	-	-	-	+	+	-
L412	Ao Ee	++	-	-	-	-	-	-	++	+(+)	-
BWR	Ao -	-	-	-	-	-	-	-	-	-	-
301	Ao -	-	-	-	-	-	-	-	-	-	-

Serum ex 1416

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex										SC
				191		306		8595		12P22		140		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
191	Ao Ka	++	+	-	-	+(+)	(+)	-	-	-	-	+(+)	-	-
306	Ao -	-	-	-	-	-	-	-	-	-	-	-	-	-
8595	Aa Ka	++	++	++	++	++	++	-	-	++	++	++	(+)	-
12P22	Ao Ka	++	++	-	-	++	++	-	-	-	-	++	(+)	-
140	Aa -	++	++	++	+(+)	++	+	-	-	++	+	-	-	-

Serum ex 2378

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex				SC
			140	306	8595	12P22	
140	-	-	-	-	-	-	-
306	Eb	+	+	-	-	-	-
8595	Eb	++	++	-	-	-	-
12P22	Eb	++	+	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				140		306		8595		12P22		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
140	-	-	-	-	-	-	-	-	-	-	-	-
306	Eb	++	++	++	++	-	-	-	-	-	-	-
8595	Eb	++	++	++	++	-	-	-	-	-	-	-
12P22	Eb	++	++	++	++	-	-	-	-	-	-	-

Serum ex 6420

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex				SC
			BWR	12P22	306	191	
BWR	Eb Fa Ha Lf	++	-	-	-	++	-
12P22	Eb - Ha Lf	++	-	-	-	++	-
306	Eb Fa - -	++	-	-	-	++	-
191	- - - -	-	-	-	-	-	-

Appendix II

Table 3 (Contd.)

Serum ex 3907

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex 311	308	BWR	L412	191	SC
311	Aa -	++	-	+(+)	+(+)	+(+)	+(+)	-
308	Ao -	-	-	-	-	-	-	-
BWR	Ao -	+(+)	(+)	-	-	(+)	-	-
L412	Ao -	-	-	-	-	-	-	-
191	Ao -	+(+)	(+)	-	-	-	-	-

Serum ex 4219

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	1:2	Serum Absorbed Red Cells ex 311 1:1	1:2	308 1:1	1:2	BWR 1:1	1:2	L412 1:1	1:2	191 1:1	1:2	SC
311	- Ee	+(+)	+	-	-	-	-	+(+)	-	-	-	-	-	-
308	Ea Ee	V	++	V	++	-	-	V	++	V	++	V	++	-
BWR	- -	-	-	-	-	-	-	-	-	-	-	-	-	-
L412	- Ee	++	+(+)	-	-	-	-	++	(+)	-	-	-	-	-
191	- Ee	++	+(+)	-	-	-	-	++	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	1:2	Serum Absorbed Red Cells ex 311 1:1	1:2	308 1:1	1:2	BWR 1:1	1:2	L412 1:1	1:2	191 1:1	1:2	SC
311	- Ee	(+)	-	-	-	-	-	-	-	-	-	-	-	-
308	Ea Ee	++	++	++	++	-	-	++	++	++	++	++	++	-
BWR	- -	-	-	-	-	-	-	-	-	-	-	-	-	-
L412	- Ee	+	-	-	-	-	-	-	-	-	-	-	-	-
191	- Ee	+(+)	-	-	-	-	-	-	-	-	-	-	-	-

Serum ex 3337

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	1:2	Serum Absorbed Red Cells ex 311 1:1	1:2	8595 1:1	1:2	308 1:1	1:2	BWR 1:1	1:2	191 1:1	1:2	SC
311	Aa - La	+(+)	+(+)	-	-	+(+)	+	-	-	+(+)	+	-	-	-
8595	Aa - -	-	-	-	-	-	-	-	-	-	-	-	-	-
308	Ao Ea La	++	++	++	++	++	++	-	-	++	++	++	++	-
BWR	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-
191	Ao - La	++	+(+)	-	-	++	+	-	-	++	+	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	1:4	Serum Absorbed Red Cells ex 311 1:2	1:4	8595 1:2	1:4	308 1:2	1:4	BWR 1:2	1:4	191 1:2	1:4	SC
311	Aa - La	++	++	-	-	++	+(+)	++	++	++	++	++	++	-
8595	Aa - -	++	++	-	-	-	-	++	++	++	++	++	++	-
308	Ao Ea La	++	++	++	++	++	++	-	-	++	++	++	++	-
BWR	Ao - -	-	-	-	-	-	-	-	-	-	-	-	-	-
191	Ao - La	++	++	-	-	++	+	-	-	++	+	-	-	-

Appendix II

Table 3 (Contd.)

Serum ex 4450

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	308	BWR	306	L412	
311	Aa -	(+)	-	-	-	-	-	-
308	Ao -	-	-	-	-	-	-	-
BWR	Ao Fa	+(+)	+	+	-	-	+	-
306	Ao Fa	++	+	+(+)	-	-	+	-
L412	Ao -	-	-	-	-	-	-	-

Serum ex 3212

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	BWR	12P22	306	191	
311	- -	-	-	-	-	-	++	-
BWR	Eb Fa	++	++	-	-	-	++	-
12P22	Eb -	++	++	-	-	-	++	-
306	Eb Fa	++	++	-	-	-	-	-
191	- -	-	-	-	-	-	-	-

Serum ex 0965

DA

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				311		308		12P22		306		
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	
311	Aa -	+	+	-	-	+	-	+	-	+	-	-
308	Ao Ea	++	+(+)	++	(+)	-	-	-	-	++	+	-
12P22	Ao Ea	++	+(+)	++	(+)	-	-	-	-	++	+	-
306	Ao -	-	-	-	-	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				311		308		12P22		306		
		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2	
311	Aa -	++	++	-	-	++	++	++	++	++	++	-
308	Ao Ea	++	+(+)	+(+)	+	-	-	-	-	+(+)	(+)	-
12P22	Ao Ea	++	+(+)	+(+)	(+)	-	-	-	-	++	(+)	-
306	- -	-	-	-	-	-	-	-	-	-	-	-

Serum ex 6010

IST

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex				SC
			311	308	12P22	191	
311	Aa -	++	-	++	++	++	-
308	Ao Ea	+	+	-	-	(+)	-
12P22	Ao Ea	+(+)	+	-	-	+	-
191	Ao -	-	-	-	-	-	-

Appendix II

Table 3 (Contd.)

Serum ex 5908

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	BWR	12P22	306	191	
311	Aa - - -	++	-	++	++	++	++	-
BWR	Ao Fa Ha Lf	++	+(+)	-	+	-	+(+)	-
12P22	Ao - Ha Lf	-	-	-	-	-	-	-
306	Ao Fa - -	++	+(+)	-	+(+)	-	+	-
191	Ao - - -	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	BWR	12P22	306	191	
311	Aa - - -	++	-	++	++	++	++	-
BWR	Ao Fa Ha Lf	++	++	-	+(+)	-	+(+)	-
12P22	Ao - Ha Lf	-	-	-	-	-	-	-
306	Ao Fa - -	++	++	-	+(+)	-	+(+)	-
191	Ao - - -	-	-	-	-	-	-	-

Serum ex 5214

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	BWR	12P22	306	L412	
311	- - - -	-	-	-	-	-	-	-
BWR	Eb Fa Ha Lf	+(+)	+	-	-	-	+	-
12P22	Eb - Ha Lf	+(+)	+(+)	-	-	-	+	-
306	Eb Fa - -	+(+)	+	-	-	-	+(+)	-
L412	- - - -	-	-	-	-	-	-	-

Serum ex 5598

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			311	140	308	12P22	191	
311	Aa -	++	-	-	++	++	++	-
140	Aa Ea	++	+(+)	-	+	+	++	-
308	Ao Ea	++	+(+)	-	-	-	+(+)	-
12P22	Ao Ea	++	+(+)	-	-	-	+(+)	-
191	Ao -	-	-	-	-	-	-	-

Serum ex 2954

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex						SC
			311	308	BWR	L412	191	12P22	
311	Aa -	++	-	++	++	+(+)	+(+)	+(+)	-
308	Ao -	+	-	-	-	-	-	-	-
BWR	Ao -	-	-	-	-	-	-	-	-
L412	Ao -	+	-	-	-	-	-	-	-
191	Ao -	++	-	-	-	-	-	-	-
12P22	Ao -	+	-	-	-	-	-	-	-

Table 3 (Contd.)

Serum ex 2330

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Unabsorbed Serum 1:2	Serum Absorbed Red Cells ex BWR 1:1	BWR 1:2	12P22 1:1	12P22 1:2	306 1:1	306 1:2	L412 1:1	L412 1:2	SC
BWR	Eb Fa Ha Lf	++	+(+)	-	-	-	-	-	-	++	+	-
12P22	Eb - Ha Lf	++	+(+)	-	-	-	-	-	-	++	+	-
306	Eb Fa - -	++	+(+)	-	-	-	-	-	-	++	(+)	-
L412	- - - -	-	-	-	-	-	-	-	-	-	-	-

Serum ex 2122

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Unabsorbed Serum 1:2	Serum Absorbed Red Cells ex 311 1:1	311 1:2	BWR 1:1	BWR 1:2	12P22 1:1	12P22 1:2	306 1:1	306 1:2	L412 1:1	L412 1:2	SC
311	Aa - - - -	++	+(+)	-	-	++	+	+(+)	+	+(+)	+	++	+	-
BWR	Ao Eb Fa Ha Lf	++	(+)	+(+)	-	-	-	-	-	-	-	+(+)	-	-
12P22	Ao Eb - Ha Lf	++	(+)	+(+)	-	-	-	-	-	-	-	+(+)	-	-
306	Ao Eb Fa - -	++	+	+(+)	-	-	-	-	-	-	-	+(+)	-	-
L412	Ao - - - -	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix II

Table 4

Sow No.	Date of Birth	Age when sampled	Breed	Iso-antibodies present
2123	20/ 4/67	1y 3m	LW	anti-Ed
564	12/ 9/65	3y -	LR	anti-Ea
7229	29/12/66	1y 10m	LW	anti-Eb
3733	28/11/64	4y -	LR	anti-Eb
6276	22/ 9/67	1y 2m	LR	anti-Ee
1416	9/ 2/62	6y 10m	LW	anti-Ea
2378	9/ 3/67	1y 7m	LR	anti-Eb
6420	25/12/64	4y 1m	LR	anti-Eb
3907	24/ 4/67	1y 11m	W	X
4219	11/ 5/68	1y -	LR	anti-Ea + anti-Ee
3337	27/ 7/67	1y 7m	LW	anti-Ea + anti-La
4450	29/10/66	2y 4m	LR	anti-Fa
3212	19/ 5/68	1y 1m	LW	anti-Eb
0965	25/ 2/66	3y 1m	LW	anti-Ea
1008	12/ 9/65	3y 5m	W	anti-Ea
5906	29/12/69	1y 5m	LR	anti-Fa
5214	14/ 3/68	1y 4m	LR	anti-Eb
5598	17/ 7/67	2y 1m	LR	anti-Ea
2954	23/ 1/67	2y 6m	W	X
3330	24/ 6/68	1y 3m	LW	anti-Eb
2122	14/ 9/67	1y 1m	LW	anti-Eb

LW - Large White LR - Landrace W - Welsh

Table 5 Log_2 of the reciprocals of the 50% end point titres of the antibodies to the antigens present on the red cells of a panel of five animals in the sera of sows which have produced litters affected with thrombocytopenic purpura.

Sow No.	Donors of Test Red Cells												Relevant Antigens
	W3184		72		T38P12		RW		L413		305		
	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	DA	IST	
P33/5/66	3.71	8.50	2.00	7.71	1.125	0.00	1.33	3.50	0.00	7.50	-	-	Aa + X + Lg
P95/8/66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	Neg.
P53/11/66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	Neg.
P13/1/67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	Neg.
P61/5/67	2.00	9.50	1.33	9.29	0.00	0.00	0.875	0.00	0.00	0.00	-	-	Aa
P13/7/67	2.33	6.14	3.33	6.33	4.57	2.50	4.11	2.50	3.83	2.50	-	-	Aa + Eb
P30/11/67	3.125	4.50	0.00	0.00	2.29	4.50	0.00	0.00	0.00	0.00	-	-	Ea
30/12/67	1.875	10.50	2.22	9.71	0.875	0.00	0.875	0.00	0.00	0.00	-	-	Aa
P11/6/68	0.00	0.00	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Neg.
P71/6/68	6.83	8.50	-	-	6.00	6.50	6.00	7.00	6.375	6.50	6.375	7.50	Aa + Eb
P30/7/68	0.00	0.00	-	-	0.00	0.00	0.00	0.00	-	-	0.00	0.00	Neg.
P81/7/68	2.875	5.00	-	-	2.71	2.50	0.00	0.00	-	-	2.50	4.50	Aa + Ea
P43/9/68	1.71	6.33	-	-	0.00	0.00	-	-	0.00	0.00	1.50	5.40	Aa
P136/10/68	0.67	7.29	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.67	7.50	Aa
P43/12/68	0.00	0.00	-	-	0.875	3.00	0.67	3.29	0.875	3.29	0.00	0.00	Eb.
P83/3/69	-	-	-	-	3.67	5.00	0.875	0.71	0.00	0.00	0.00	0.00	Ea
P94/4/69	-	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	1.33	7.50	Aa
P116/7/69	-	-	-	-	1.71	3.50	0.00	0.00	0.875	1.71	0.00	0.00	Ea + X
AB 1	3.125	9.50	-	-	1.67	0.00	1.875	0.00	0.00	0.00	-	-	Aa + Ea + Eb
AB 2	4.33	8.50	-	-	1.33	0.00	0.00	0.00	0.00	0.00	-	-	Aa + Ea
AB 3	0.875	7.50	-	-	0.00	0.00	0.00	0.00	0.00	0.00	-	-	Aa
AB 4	1.875	0.00	0.00	0.00	1.71	1.50	1.875	1.29	1.875	0.00	-	-	Ee
AB 5	2.40	9.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	Aa
E 305	3.71	4.80	0.00	0.00	3.33	4.20	0.00	0.00	0.00	0.00	-	-	Ea

X - Unidentifiable

Appendix II

Table 6 Identification of iso-antibodies present in the sera of sows which have produced litters affected with thrombocytopenic purpura (Appendix II, Table 5) by checkerboard analysis.

Serum ex P33/5/66

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			W3184	72	T38P12	RW	L413	
W3184	Aa -	V	-	-	V	V	V	-
72	Aa -	++	-	-	~~V~~	V	V	-
T38P12	Ao Ka	+(+)	-	-	-	-	(+)	-
RW	Ao Ka	++	-	-	-	-	-	-
L413	Ao -	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex										SC
				W3184		72		T38P12		RW		L413		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa Lg	++	++	-	-	-	-	++	++	++	++	++	++	-
72	Aa Lg	++	++	-	-	-	-	++	++	++	++	++	++	-
T38P12	Ao -	-	-	-	-	-	-	-	-	-	-	-	-	-
RW	Ao Lg	++	++	-	-	-	-	++	++	-	-	-	-	-
L413	Ao Lg	++	++	-	-	-	-	++	++	-	-	-	-	-

Serum ex P13/7/67

DA

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex										SC
				W3184		72		T38P12		RW		L413		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa -	V	++	-	-	-	-	++	-	++	-	+	-	-
72	Aa Eb	++	++	V	++	-	-	++	-	++	-	+	-	-
T38P12	Ao Eb	V	V	V	++	-	-	-	-	-	-	-	-	-
RW	Ao Eb	V	++	V	++	-	-	-	-	-	-	-	-	-
L413	Ao Eb	V	++	V	++	-	-	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex										SC
				W3184		72		T38P12		RW		L413		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa -	++	++	-	-	-	-	++	++	++	++	++	++	-
72	Aa Eb	++	++	+	-	-	-	++	++	++	++	++	++	-
T38P12	Ao Eb	+(+)	(+)	+	-	-	-	-	-	-	-	-	-	-
RW	Ao Eb	+(+)	(+)	+	-	-	-	+	(+)	-	-	-	-	-
L413	Ao Eb	+(+)	(+)	+	-	-	-	-	-	-	-	-	-	-

Serum ex P30/11/67

DA

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				W3184		72		T38P12		RW		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Aa Ea	V	+(+)	-	-	++	-	-	-	++	-	-
72	Aa -	-	-	-	-	-	-	-	-	-	-	-
T38P12	Ao Ea	+(+)	+	-	-	++	-	-	-	++	-	-
RW	Ao -	-	-	-	-	-	-	-	-	-	-	-

Appendix II

Table 6 (Contd.)

Serum ex P30/11/67

IST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	Unabsorbed Serum 1:4	Serum Absorbed Red Cells ex W3184 1:2	W3184 1:4	72 1:2	72 1:4	T38P12 1:2	T38P12 1:4	RW 1:2	RW 1:4	SC
W3184	Aa Ea	++	++	-	-	++	++	-	-	++	++	-
72	Aa -	-	-	-	-	-	-	-	-	-	-	-
T38P12	Ao Ea	++	++	-	-	++	++	-	-	++	+(+)	-
RW	Ao -	-	-	-	-	-	-	-	-	-	-	-

Serum ex P71/6/68

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:4	Unabsorbed Serum 1:8	Serum Absorbed Red Cells ex W3184 1:4	W3184 1:8	305 1:4	305 1:8	T38P12 1:4	T38P12 1:8	RW 1:4	RW 1:8	L413 1:4	L413 1:8	SC
W3184	Aa -	V	V	-	-	+	-	++	++	++	++	++	++	-
305	Aa -	V	V	-	-	-	-	++	++	V	++	V	++	-
T38P12	Ao Eb	V	V	V	++	V	++	-	-	+	-	+	-	-
RW	Ao Eb	V	++	++	++	++	++	+	-	-	-	-	-	-
L413	Ao Eb	V	V	++	++	++	++	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:16	Unabsorbed Serum 1:32	Serum Absorbed Red Cells ex W3184 1:16	W3184 1:32	305 1:16	305 1:32	T38P12 1:16	T38P12 1:32	RW 1:16	RW 1:32	L413 1:16	L413 1:32	SC
W3184	Aa -	++	++	-	-	-	-	++	++	++	++	++	++	-
305	Aa -	++	++	-	-	-	-	++	++	++	++	++	++	-
T38P12	Ao Eb	++	++	++	-	++	+	-	-	-	-	-	-	-
RW	Ao Eb	++	++	++	(+)	++	(+)	-	-	-	-	-	-	-
L413	Ao Eb	++	++	++	+	++	+	-	-	-	-	-	-	-

Serum ex P81/7/68

DA

Cell Source	Relevant Antigens	Unabsorbed Serum 1:2	Unabsorbed Serum 1:4	Serum Absorbed Red Cells ex W3184 1:2	W3184 1:4	305 1:2	305 1:4	T38P12 1:2	T38P12 1:4	8 1:2	8 1:4	RW 1:2	RW 1:4	SC
W3184	Aa Ea	V	+(+)	-	-	+	-	++	+	+(+)	-	+(+)	+	-
305	Aa -	++	+	-	-	-	-	+(+)	+	+(+)	-	+(+)	(+)	-
T38P12	Ao Ea	++	+	-	-	+(+)	-	-	-	-	-	+	-	-
8	Ao Ea	+(+)	+	-	-	+(+)	(+)	-	-	-	-	+	-	-
RW	Ao -	-	-	-	-	-	-	-	-	-	-	-	-	-

Serum ex P43/12/68

IST

Cell Source	Relevant Antigens	Unabsorbed Serum 1:1	Unabsorbed Serum 1:2	Serum Absorbed Red Cells ex W3184 1:1	W3184 1:2	T38P12 1:1	T38P12 1:2	RW 1:1	RW 1:2	L413 1:1	L413 1:2	305 1:1	305 1:2	SC
W3184	Aa -	-	-	-	-	-	-	-	-	-	-	-	-	-
T38P12	Ao Eb	++	++	++	+	-	-	-	-	-	-	++	+	-
RW	Ao Eb	++	++	++	+(+)	-	-	-	-	-	-	++	+	-
L413	Ao Eb	++	++	++	+(+)	-	-	-	-	-	-	++	+(+)	-
305	Aa -	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix II

Table 6 (Contd.)

Serum ex AB.4

DA

Cell Source	Relevant Antigens	Unabsorbed Serum	Serum Absorbed Red Cells ex					SC
			W3184	72	T38P12	RW	L413	
W3184	Ee	V	-	++	-	-	-	-
72	-	-	-	-	-	-	-	-
T38P12	Ee	++	-	++	-	-	-	-
RW	Ee	V	-	++	-	-	-	-
L413	Ee	V	-	++	-	-	-	-

Serum ex E.305

DA

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				W3184		72		T38P12		RW		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Ea	V	++	-	-	++	+	-	-	++	+	-
72	-	-	-	-	-	-	-	-	-	-	-	-
T38P12	Ea	V	++	-	-	++	+	-	-	++	+	-
RW	-	-	-	-	-	-	-	-	-	-	-	-

IST

Cell Source	Relevant Antigens	Unabsorbed Serum		Serum Absorbed Red Cells ex								SC
				W3184		72		T38P12		RW		
		1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	1:2	1:4	
W3184	Ea	++	++	-	-	++	+(+)	-	-	++	+(+)	-
72	-	-	-	-	-	-	-	-	-	-	-	-
T38P12	Ea	++	++	-	-	++	+(+)	-	-	++	+(+)	-
RW	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX III

Appendix III

Table 1 Red cell counts (x 10^6 per cu. mm.), packed cell volume and haemaglobin estimation (g per 100 ml.) of piglets in litters ex sows 88, 405, 301 and 302.

Dam	Piglet No.	Ea Type	Bleed 1			Bleed 2			Bleed 3			Bleed 4			Bleed 5			Bleed 6		
			Rbc. x 10^6	PCV	Hbg.%	Rbc.x 10^6	PCV	Hbg.%	Rbc.x 10^6	PCV	Hbg.%	Rbc.x 10^6	PCV	Hbg.%	Rbc.x 10^6	PCV	Hbg.%	Rbc.x 10^6	PCV	Hbg.%
88	56	+	6.07	41	13.8	5.04	31	9.5	3.41	30	8.6	5.41	38	13.6	5.07	38	10.8	5.66	37	11.6
	57	+	6.25	38	13.2	4.31	28.5	8.4	3.08	23	6.9	4.40	35	12.6	4.61	38	9.8	5.84	39	11.7
	58	+	4.65	32	9.8	4.03	22	6.4	3.37	27	7.8	5.49	30	11.2	4.77	38	9.3	5.69	40	11.6
	59	+	6.66	46	15.4	4.30	30	8.8	3.24	25.5	7.8	5.49	39	13.6	5.40	40.5	10.8	6.36	39	12.6
	60	-	6.45	46	14.0	3.65	24	6.4	2.48	20	6.5	4.10	32	12.6	4.20	36.5	9.8	5.96	37	11.2
	61	+	5.99	42	14.6	4.97	34	10.3	3.19	23	7.8	5.06	35	12.5	5.04	39	9.8	6.23	40	12.6
	62	-	6.03	44	13.8	5.96	41	13.2	1.95	18	5.1	4.22	30	11.7	4.64	37	9.3	5.48	38	11.6
	63	+	5.40	39	12.2	2.81	21	5.6	2.75	26	6.9	4.81	34	11.7	4.29	37.5	9.3	5.63	38	11.7
	64	+	5.63	42	13.8	5.30	35	10.8	4.39	29	10.2	4.70	33	12.1	5.23	37	9.8	5.80	37	11.7
	65	+	5.93	45	14.6	3.69	28	7.8	2.52	19	6.6	5.05	35	12.5	4.96	40	10.3	6.03	39	13.1
405	29	+	6.14	47	14.5	5.04	36	10.7	4.00	29	8.8	4.07	34	9.8	5.12	38	12.2	5.44	38	12.2
	30	+	6.07	41	13.2	4.35	29	8.8	3.47	23	7.0	4.41	34	10.0	5.83	38	11.9	6.07	38	12.8
	33	+	6.01	43	13.6	4.35	32	9.5	4.00	28	8.3	3.90	31	8.8	5.18	38	11.9	6.15	40	12.8
	34	+	6.18	40	12.7	3.96	26	8.3	3.50	23	7.4	4.24	32	8.6	5.37	39	12.2	6.08	38	12.2
	35	+	7.30	48	15.5	5.64	38	11.9	5.03	32	10.0	5.07	34	10.7	5.83	39	12.7	6.13	38	12.2
	37	+	6.71	49	15.0	4.91	35	10.3	4.55	31	9.8	4.22	32	9.8	4.91	34	10.8	6.08	38	11.7
	38	+	5.83	40	12.7	3.92	28	7.8	3.56	24	7.9	3.99	33	9.2	5.20	40	11.9	6.17	40	13.0
	39	-	6.27	43	14.5	4.39	32	9.3	3.67	26	8.5	4.21	33	8.8	5.20	40	12.2	5.69	39	12.2
	40	-	5.78	43	15.0	4.40	32	9.3	3.78	28	9.2	3.69	33	9.2	4.33	36	11.2	5.60	40	12.8
301	321	-	4.98	39	11.2	3.83	27	8.3	3.36	30	9.2	3.98	31	9.8	4.89	38	10.9	5.61	39	12.2
	322	-	5.16	36	11.2	3.27	23	6.5	3.19	30	8.3	3.56	31	8.6	4.67	38	10.7	5.36	37	11.9
	323	+	5.44	41	13.2	3.65	25	7.4	3.20	27	8.3	3.58	27	7.8	3.93	35	9.3	5.19	40	12.2
	324	-	5.43	44	13.3	4.09	30	9.8	3.21	28	7.8	3.59	28	8.3	4.19	37	11.2	4.69	36	11.2
	327	-	6.01	46	13.8	4.22	27	8.3	3.76	31	8.8	3.43	29	8.8	4.32	36	10.7	5.10	38	11.9
	328	+	5.55	44	13.2	3.95	27	8.3	3.88	29	8.8	3.69	30	8.8	5.70	41	12.6	6.00	43	14.8
	329	+	4.87	40	12.2	4.14	30	9.8	3.59	29	8.3	3.40	29	8.8	5.02	39	11.2	5.56	38	12.6
	330	+	4.64	39	11.2	3.48	26	7.8	3.46	29	8.8	3.34	30	8.8	4.57	36	10.7	5.18	35	10.7
302	333	+	5.87	45	13.8	4.57	29	9.3	4.06	29	8.8	4.00	31	9.3	4.87	38	11.2	4.83	36	11.9
	334	+	5.44	42	12.2	4.48	27	8.8	3.77	28	7.9	4.42	31	9.3	4.88	36	10.3	5.69	38	12.2
	335	-	5.97	41	12.7	2.62	18	5.6	4.39	34	9.0	4.69	34	9.8	5.15	38	10.7	5.71	37	12.6
	336	+	5.21	37	11.2	3.98	27	8.3	3.65	29	7.4	4.36	30	9.3	4.75	38	9.8	5.29	36	12.2
	337	+	5.58	39	11.7	3.50	23	7.0	3.47	28	7.4	3.56	30	9.3	4.64	37	10.7	4.82	35	11.2
	338	+	5.64	44	13.6	4.59	30	9.3	3.96	31	8.8	4.92	32	9.8	4.51	37	10.3	5.53	40	13.2
	339	-	4.51	35	10.3	3.42	24	7.0	3.37	27	7.0	4.92	32	10.2	5.31	40	11.2	5.59	39	13.2
	340	+	6.31	48	14.0	4.64	32	10.3	4.06	36	8.8	4.41	32	9.8	5.02	40	11.2	5.23	37	11.8
	341	+	4.94	37	10.7	3.61	24	7.9	3.02	24	6.3	3.24	28	7.8	3.75	33	8.8	4.89	44	12.3

APPENDIX IV

Appendix IV

Table 1

	Reciprocal Dilutions of Haemolysin							
	100	500	1000	2000	5000	7500	10,000	15,000
	4	4	4	4	3	2	2	1
	4	4	4	4	3	2	2	1
	4	4	4	4	3	2	2	1
Controls	0	0	0	0	0	0	0	0

Titration of haemolysin; guinea pig complement diluted 1 in 20.

Table 2

	Reciprocal Dilutions of Complement									
	100	120	140	160	180	200	250	300	350	400
	4	4	4	4	4	4	3	3	2	2
	4	4	4	4	4	4	3	3	2	2
	4	4	4	4	4	4	3	3	3	2
Controls	0	0	0	0	0	0	0	0	0	0

Titration of guinea pig complement; sheep red cells sensitised with an equal volume of haemolysin diluted 1 in 1000.

Table 3

Serum ex R17	Reciprocal Dilutions of Serum										Antigen controls		
	4	8	16	32	64	128	256	512	1024	2048			
Preimmunisation	4	4	4	4	4	4	4	4	4	4			
Hyperimmune	0	0	0	0	2	4	4	4	4	4			
Serum Controls (5 uC^{1}	4	4	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (2 uC^{1}	4	4	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (1 uC^{1}	2	2	2	2	2	2	2	2	2	2	2	2	2

Complement Fixation Test: Serum ex rabbit 17 (heated); porcine platelets (300 x 10^{3} per cu. mm.); Overnight fixation at 4°C.

Table 4

Reciprocal Dilution of Antiserum	Concentration of Platelets x 10^{4}									Controls
	40	35	30	25	20	15	10	5	2.5	
4	0	0	0	0	0	0	0	0	2	4
8	0	0	0	0	0	0	0	0	2	4
16	0	0	0	0	0	0	0	0	4	4
32	tr	0	0	0	0	0	0	2	4	4
64	4	2	2	2	0	0	2	4	4	4
128	4	4	4	4	3	2	4	4	4	4
256	4	4	4	4	4	4	4	4	4	4
512	4	4	4	4	4	4	4	4	4	4
1024	4	4	4	4	4	4	4	4	4	4
2048	4	4	4	4	4	4	4	4	4	4

Titration of antigen (porcine platelets) with hyperimmune serum ex R17. Overnight fixation at 4°C.

Appendix IV

Table 5

Serum ex 228	Reciprocal dilutions of Serum								Antigen controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Hyperimmune	4	4	0	0	2	4	4	4			
Serum Controls (3 uC^1)	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (2 uC^1)	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (1 uC^1)	4	4	3	2	2	2	2	2	2	2	2

Complement Fixation Test: Serum ex 228 (heated); bovine platelets (150 x 10^3 per cu. mm.); overnight fixation at 4°C.

Table 6

Serum ex 228	Reciprocal dilutions of Serum								Antigen controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Hyperimmune	4	4	0	2	4	4	4	4			
Serum Controls (3 uC^1)	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (2 uC^1)	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (1 uC^1)	4	4	3	2	2	2	2	2	2	2	2

Complement Fixation Test: Serum ex 228 (heated); bovine platelets (150 x 10^3 per cu. mm.); fixation at 20°C for 3 hours.

Table 7

Serum ex 228	Reciprocal dilutions of Serum								Antigen controls		
	5	10	20	40	80	160	320	640			
Preimmunisation	4	4	4	4	4	4	4	4			
Hyperimmune	0	0	2	4	4	4	4	4			
Serum Controls (5 uC^1)	4	4	4	4	4	4	4	4	4	4	4
Serum Controls (2 uC^1)	2	2	4	4	4	4	4	4	4	4	4
Serum Controls (1 uC^1)	0	0	1	1	2	2	2	2	2	2	2

Complement Fixation Test: Serum ex 228 (inactivated by heat and formalin); bovine platelets (150 x 10^3 per cu. mm.); fixation at 4°C overnight.

Appendix IV

Table 8

Serum ex 228	Reciprocal dilutions of Serum								Antigen Controls
	5	10	20	40	80	160	320	640	
Preimmunisation	4	4	4	4	4	4	4	4	
Hyperimmune	0	0	2	4	4	4	4	4	
Serum Controls (5 uC^1	4	4	4	4	4	4	4	4	4 4 4
Serum Controls (2 uC^1	2	2	4	4	4	4	4	4	4 4 4
Serum Controls (1 uC^1	0	0	1	1	2	2	2	2	2 2 2

Complement Fixation Test: Serum ex 228 (inactivated by heat and formalin); bovine platelets (150 x 10^3 per cu. mm.); fixation at 4°C overnight.

Table 9

Serum ex 228	Reciprocal dilutions of Serum								Antigen controls
	5	10	20	40	80	160	320	640	
Preimmunisation	4	4	4	4	4	4	4	4	
Hyperimmune	0	2	4	4	4	4	4	4	
Serum Controls (5 uC^1	4	4	4	4	4	4	4	4	4 4 4
Serum Controls (2 uC^1	2	3	4	4	4	4	4	4	4 4 4
Serum Controls (1 uC^1	0	0	1	2	2	2	2	2	2 2 2

Complement Fixation Test: Serum ex 228 (inactivated by heating and formalin); bovine platelets (150 x 10^3 per cu. mm.); fixation at 20°C for 3 hours.

Table 10

Serum ex 228	Reciprocal dilutions of Serum								Antigen Controls
	5	10	20	40	80	160	320	640	
Preimmunisation	4	4	4	4	4	4	4	4	
Hyperimmune	0	2	4	4	4	4	4	4	
Serum Controls (5 uC^1	4	4	4	4	4	4	4	4	4 4 4
Serum Controls (2 uC^1	2	3	3	4	4	4	4	4	4 4 4
Serum Controls (1 uC^1	0	0	1	1	2	2	2	2	2 2 2

Complement Fixation Test: Serum ex 228 (inactivated by heating and formalin); bovine platelets (150 x 10^3 per cu.mm.); fixation at 37°C for 1½ hours.

Appendix IV

Table 11

APG	Reciprocal of Dilutions										
	4	8	16	32	64	128	256	512	1024	2048	4096
R 1	++	++	++	++	++	++	++	+(+)	-	-	-
R 6	++	++	++	++	++	++	++	++	++	(+)	-
R29	++	++	++	++	++	++	++	++	++	++	(+)
R30	++	++	++	++	++	++	++	+	-	-	-

Titration of rabbit anti-pig globulin sera.

Table 12a

Reciprocal Dilutions of R8	Reciprocal Dilutions of Reagent 30										
	5	10	20	30	40	50	60	80	100	150	SC
4	++	++	++	++	++	++	++	++	+(+)	+	-
8	++	++	++	++	++	++	++	++	++	+	-
16	++	++	++	++	++	++	++	++	++	+(+)	-
32	++	++	++	++	++	++	++	++	++	++	-
64	++	++	++	++	++	++	++	++	++	++	-
128	++	++	++	++	++	++	++	++	++	+(+)	-
256	++	++	++	++	++	++	++	++	++	+(+)	-
512	++	++	++	++	++	++	++	+(+)	+(+)	+	-
1024	++	++	++	+(+)	++	++	+(+)	+(+)	+	-	-
2048	+(+)	+(+)	+(+)	+	+	(+)	(+)	(+)	-	-	-
SC	-	-	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 30 and rabbit anti-pig-globulin, R8.

Table 12b

Reciprocal Dilutions of R8	Reciprocal Dilutions of Reagent 50										
	5	10	20	40	80	160	320	640	1280	2560	SC
4	++	++	++	++	++	++	+	-	-	-	-
8	++	++	++	++	++	++	++	+	-	-	-
16	++	++	++	++	++	++	++	(+)	-	-	-
32	++	++	++	++	++	++	++	++	-	-	-
64	++	++	++	++	++	++	++	++	-	-	-
128	++	++	++	++	++	++	++	+(+)	-	-	-
256	++	++	++	++	++	++	++	-	-	-	-
512	++	++	++	++	++	++	+	-	-	-	-
1024	++	++	++	++	+(+)	+(+)	-	-	-	-	-
1068	+(+)	+(+)	+(+)	-	-	-	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 59 and APG R8.

Table 12c

Reciprocal Dilutions of R8	Reciprocal Dilutions of Reagent 75								
	5	10	20	40	80	160	320	640	SC
4	++	++	++	++	+	-	-	-	-
8	++	++	++	++	++	+	-	-	-
16	++	++	++	++	++	+	-	-	-
32	++	++	++	++	++	+(+)	-	-	-
64	++	++	++	++	++	+(+)	-	-	-
128	++	++	++	++	++	+	-	-	-
256	++	++	++	++	++	+	-	-	-
512	++	++	++	++	+(+)	(+)	-	-	-
1024	++	++	++	+	+	-	-	-	-
2048	+	+	-	-	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 75 and APG R8.

Appendix IV

Table 13a

Reciprocal Dilutions of R29	Reciprocal Dilutions of Reagent 30										
	5	10	20	40	80	160	320	640	1280	2560	SC
8	++	++	++	++	++	+(+)	-	-	-	-	-
16	++	++	++	++	++	++	(+)	-	-	-	-
32	++	++	++	++	++	++	(+)	-	-	-	-
64	++	++	++	++	++	++	+	-	-	-	-
128	++	++	++	++	++	++	++	-	-	-	-
256	++	++	++	++	++	++	++	-	-	-	-
512	++	++	++	++	++	++	++	-	-	-	-
1024	++	++	++	++	++	++	++	+	-	-	-
2048	++	++	++	++	++	++	+(+)	-	-	-	-
4096	+(+)	+(+)	+	+	(+)	(+)	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 30 and APG, R29.

Table 13b

Reciprocal Dilutions of R29	Reciprocal Dilutions of Reagent 59										
	5	10	20	40	80	160	320	640	1280	2560	SC
8	++	++	++	++	+	-	-	-	-	-	-
16	++	++	++	++	+(+)	-	-	-	-	-	-
32	++	++	++	++	++	(+)	-	-	-	-	-
64	++	++	++	++	++	++	(+)	-	-	-	-
128	++	++	++	++	++	++	(+)	-	-	-	-
256	++	++	++	++	++	++	+(+)	-	-	-	-
512	++	++	++	++	++	++	++	-	-	-	-
1024	++	++	++	++	++	++	++	-	-	-	-
2048	++	++	++	++	++	++	+(+)	-	-	-	-
4096	++	++	+(+)	(+)	(+)	-	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 59 and APG, R29.

Table 13c

Reciprocal Dilutions of R29	Reciprocal Dilutions of Reagent 61								
	5	10	20	40	80	160	320	640	SC
8	++	++	++	+(+)	-	-	-	-	-
16	++	++	++	+(+)	(+)	-	-	-	-
32	++	++	++	++	+	-	-	-	-
64	++	++	++	++	+	-	-	-	-
128	++	++	++	++	+(+)	-	-	-	-
256	++	++	++	++	+	-	-	-	-
512	++	++	++	++	(+)	-	-	-	-
1024	++	++	++	++	-	-	-	-	-
2048	++	++	++	++	-	-	-	-	-
4096	++	+(+)	(+)	-	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	-

Chessboard titration of reagent 61 and APG, R29.

APPENDIX V

Appendix V

Table 1

Age at bleeding	Piglet No.							
	443	444	445	446	447	448	449	450
Birth	310	285	287	279	246	305	312	-
24h	223	240	218	255	213	343	287	217
72h	326	304	324	347	354	330	361	399
6d	510	455	528	526	504	457	618	449
8d	658	574	634	751	672	768	718	664
10d	754	496	939	1008	948	820	977	-
18d	568	558	562	620	518	573	558	491
24d	363	498	483	481	451	485	503	460
31d	-	380	431	414	504	497	455	391
38d	-	527	460	486	420	486	448	504

Thrombocyte counts x 10^3 per cu. mm. for normal litter ex 309.

Table 2

Age at bleeding	Piglet No.							
	529	530	531	532	533	534	535	536
Birth	276	272	246	290	236	200	270	223
24h	204	250	-	250	236	226	241	215
72h	403	414	-	378	355	367	387	408
5d	399	407	-	492	533	524	466	394
7d	661	556	-	591	504	502	632	486
10d	562	689	-	573	632	633	614	482
12d	493	535	-	530	487	651	489	459
14d	506	499	-	499	418	651	379	557
17d	405	499	-	572	469	585	413	507
19d	639	667	-	647	570	645	462	401
21d	665	652	-	630	540	526	486	358
40d	437	476	-	532	429	433	376	481

Thrombocyte counts x 10^3 per cu. mm. for normal litter ex 328.

Table 3

Age at bleeding	Piglet No.									
	433	434	435	436	437	438	439	440	441	442
Birth										
48h	118	155	170	156	38	20	39	122	88	28
4d	186	227	210	272	120	84	81	223	171	46
6d	335	392	356	499	248	284	208	272	334	212
9d	124	221	193	196	329	215	206	162	126	113
11d	193	162	291	171	173	287	248	235	228	190
13d	217	182	323	278	316	366	281	-	-	238
16d	273	257	287	304	321	371	236	-	-	355
18d	289	362	300	389	374	346	394	-	-	413
23d	464	413	436	414	441	397	423	-	-	451
27d	435	352	376	376	522	356	339	-	-	412

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 30 (1st litter).

Appendix V

Table 4

Age at bleeding	Piglet No.							
	572	573	574	575	576	577	578	579
Birth								
12h	-	157	161	100	118	100	134	105
36h	-	99	88	82	121	95	102	84
3½d	-	296	330	241	282	275	279	247
7d	-	241	352	240	269	275	335	328
9d	-	-	303	110	236	201	225	232
11d	-	-	192	48	180	167	144	115
12d	-	-	195	16	169	173	155	156
14d	-	-	199	-	153	138	136	50
16d	-	-	207	-	172	212	154	53
18d	-	-	251	-	258	271	208	70
22d	-	-	345	-	319	272	141	127
24d	-	-	306	-	305	280	220	176
29d	-	-	382	-	392	241	349	296

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 30 (2nd litter).

Table 5

Age at bleeding	Piglet No.							
	344	345	346	347	348	349	350	351
Birth	391	454	463	459	405	422	410	346
24h	89	64	105	159	95	108	89	96
48h	127	217	172	-	155	194	228	143
5d	420	286	509	-	481	532	515	302
9d	428	436	437	-	336	613	489	72
13d	258	258	228	-	98	193	236	-
14d	126	116	197	-	45	70	80	-
16d	198	225	179	-	65	120	177	-
19d	316	366	120	-	117	254	241	-
23d	540	572	43	-	271	287	349	-

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 343.

Table 6

Age at bleeding	Piglet No.						
	313	314	315	316	317	318	319
Birth	286	392	329	299	392	346	295
24h	77	-	34	20	13	33	42
4d	502	-	94	364	207	277	195
7d	450	-	614	399	294	497	464

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 304.

Appendix V

Table 7

Age of bleeding	Piglets ex 1083						Piglets ex 305					
	360	361	362	363	364	365	369	370	371	372	373	374
Birth	405	392	330	406	392	346	313	398	343	325	371	409
24h	339	370	382	369	346	385	81	241	89	99	241	77
4d	386	365	385	346	-	391	253	275	171	230	327	-
8d	610	293	682	565	471	717	554	429	-	453	501	-
11d	839	1110	835	860	715	765	292	523	-	336	576	-
13d	-	-	-	-	-	-	143	470	-	86	490	-
15d	437	-	603	552	426	774	208	415	-	46	425	-

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 1083 and 305.

Piglets nursed by 1083 - 361, 363, 364, 365, 370 and 373.

Piglets nursed by 305 - 360, 362, 369, 371, 372 and 374.

Table 8

Age at bleeding	Piglet No.								
	412	413	414	415	416	417	418	419	420
Birth	221	260	282	213	236	285	224	248	204
24h	189	260	221	77	34	20	33	42	247
4d	437	366	358	110	199	187	170	173	359
8d	623	587	585	431	497	529	667	533	546
14d	581	743	551	262	261	384	599	480	485
19d	612	677	637	594	412	443	-	380	611

Thrombocyte counts x 10^3 per cu. mm. of piglets ex 305 by her son, 311.

Normal piglets - 412, 413, 414 and 420.

Piglets affected with thrombocytopenic purpura - 415, 416, 417, 418 and 419.

A P P E N D I X V I

Appendix VI

Table 1

Age at bleeding	Piglet No.										
	402	403	404	405	406	407	408	409	410	411	412
Birth	310	300	415	325	-	269	249	289	-	-	-
12h	22	28	67	147	17	-	167	-	34	19	-
35h	36	-	64	132	-	62	123	42	31	-	-
5½d	-	-	258	702	-	287	779	-	-	-	-
7½d	-	-	92	1080	-	89	698	-	-	-	-
9½d	-	-	52	625	-	232	622	-	-	-	-
12½d	-	-	410	610	-	497	475	-	-	-	-
15½d	-	-	657	472	-	650	610	-	-	-	-
19½d	-	-	551	-	-	529	585	-	-	-	-
23½d	-	-	586	-	-	431	557	-	-	-	-
26½d	-	-	451	-	-	501	650	-	-	-	-
34½d	-	-	413	-	-	572	330	-	-	-	-

Thrombocyte counts x 10^3 per cu. mm. for first litter ex 307 by 311.

Table 2 Antiglobulin consumption test: serum ex 307: platelets ex piglets ex 307 by 311 (1st litter).

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Platelets ex 404	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	(+)	-	-	-
+ HI serum	++	++	++	-	-	-	-	-	-	-
Platelets ex 407	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	(+)	-	-	-
+ HI serum	++	++	++	(+)	-	-	-	-	-	-
Platelets ex 408	++	++	++	++	++	++	++	-	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	+(+)	-	-	-	-

Appendix VI

Table 3

Age at bleeding	Piglet No.						
	454	455	456	457	458	459	460
Birth	350	240	292	285	315	289	304
12h	23	163	26	242	19	41	15
3½d	249	303	243	291	-	63	144
5½d	445	463	432	423	-	133	284
7½d	458	560	590	535	-	285	400
11½d	650	699	615	651	-	520	515
15½d	510	584	537	533	-	458	544
21½d	588	684	639	606	-	634	547
24½d	524	519	521	549	-	-	511
28½d	459	434	423	479	-	-	520
31½d	522	541	531	519	-	-	499
35½d	535	425	604	487	-	-	571

Thrombocyte counts x 10^3 per cu. mm. for first litter ex 306 by 311.

Table 4 Antiglobulin consumption test: serum ex 306; platelets ex piglets ex 306 by 311 (1st litter).

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Platelets ex 454	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	(+)	-	-	-	-	-
Platelets ex 455	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 456	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	-	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-
Platelets ex 457	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 460	++	++	++	++	++	++	+	-	-	-
+ PI serum	++	++	++	++	++	++	(+)	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-

Appendix VI

Table 5

Age at bleeding	Piglet No.										
	482	483	484	485	486	487	488	489	490	491	492
Birth	-	-	152	217	283	284	285	223	199	250	-
24h	76	139	144	184	219	155	88	92	142	69	102
48h	52	189	191	188	222	253	93	65	217	108	142
8d	338	402	417	416	490	472	313	329	365	328	392
7d	404	502	473	525	498	544	534	475	459	414	384
12d	420	310	561	594	578	598	501	380	617	549	455
14d	507	348	552	558	530	404	475	430	536	555	351
19d	486	310	517	523	510	503	550	545	481	509	505
21d	421	459	506	472	440	387	507	499	442	501	461
23d	555	415	552	485	465	414	413	337	477	448	375
29d	507	439	523	565	477	454	501	399	405	451	589
35d	427	331	547	459	484	491	410	489	321	356	322

Thrombocyte counts x 10^3 per cu. mm. of piglets in 2nd litter ex 307 by 311.

Table 6 Antiglobulin consumption test. Serum ex 307, platelets ex piglets in second litter ex 307 by 311.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	-	-
Platelets ex 482	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	++	+	-	-	-	-	-
Platelets ex 483	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	++	++	++	++	+	-	-
Platelets ex 484	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	+(+)	+	-	-	-
Platelets ex 485	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-
Platelets ex 486	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	(+)	-	-	-
Platelets ex 487	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	++	(+)	-	-	-	-
Platelets ex 488	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	+	-	-	-	-	-	-
Platelets ex 489	++	++	++	++	++	++	++	-	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-
Platelets ex 490	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	(+)	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 491	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	(+)	-	-	-	-	-	-
Platelets ex 492	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	+	-	-	-	-

Appendix VI

Table 7

Age at bleeding	Piglet No.									
	493	494	495	496	497	498	499	500	501	502
Birth	333	249	398	335	211	289	261	285	341	269
24h	254	34	293	19	-	87	36	-	67	30
48h	318	-	359	-	-	107	-	-	116	-
4d	426	-	472	-	-	417	-	-	365	-
7d	513	-	548	-	-	611	-	-	554	-
9d	470	-	600	-	-	544	-	-	440	-
11d	453	-	509	-	-	529	-	-	347	-
15d	395	-	567	-	-	518	-	-	316	-
18d	398	-	488	-	-	466	-	-	416	-
21d	466	-	478	-	-	536	-	-	460	-
24d	411	-	418	-	-	531	-	-	397	-
35d	396	-	283	-	-	412	-	-	210	-

Thrombocyte counts x 10^3 per cu. mm. of piglets in 2nd litter ex 306 by 311.

Table 8 Antiglobulin consumption test. Serum ex 306; platelets ex piglets in second litter ex 306 by 311.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Platelets ex 493	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	++	(+)	-	-
+ HI serum	++	++	++	++	++	++	++	-	-	-
Platelets ex 495	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 498	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	++	(+)	-	-
+ HI serum	++	++	++	+	-	-	-	-	-	-
Platelets ex 501	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	++	-	-	-
+ HI serum	++	++	++	(+)	-	-	-	-	-	-

Appendix VI

Table 9

Age at bleeding	Piglet No.									
	580	581	582	583	584	585	586	587	588	589
48h	234	65	208	134	47	154	111	157	129	128
5d	353	312	257	325	269	329	251	318	266	188
8d	458	519	483	586	428	467	433	407	436	377
10d	435	357	411	465	422	534	352	361	294	345
12d	336	378	321	307	329	-	325	306	296	312
15d	341	351	335	315	299	-	455	329	311	262
17d	257	362	290	258	218	-	412	328	280	280
19d	243	241	270	201	311	-	444	350	272	287
22d	326	273	369	321	313	-	466	351	294	265
26d	332	262	365	298	280	-	419	332	327	333

Thrombocyte counts x 10^3 per cu. mm. of piglets in third litter ex 307 by 311.

Table 10 Antiglobulin consumption test. Serum ex 307; platelets ex piglets in third litter ex 307 by 311.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Platelets ex 580	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 581	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-
Platelets ex 582	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 583	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	(+)	-	-
+ HI serum	++	++	++	+(+)	(+)	-	-	-	-	-
Platelets ex 584	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	+(+)	-	-	-	-	-	-
Platelets ex 586	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	(+)	-	-
+ HI serum	++	++	++	+(+)	+	-	-	-	-	-
Platelets ex 587	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	++	(+)	-	-	-	-
Platelets ex 588	++	++	++	++	++	++	+(+)	-	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	+	-	-	-	-	-
Platelets ex 589	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	+	-	-	-	-	-

Appendix VI

Table 11

Age at bleeding	Piglet No.								
	563	564	565	566	567	568	569	570	571
Birth	277	259	253	236	203	260	260	217	228
24h	220	101	-	224	213	229	-	65	78
3d	365	182	260	311	351	362	-	143	162
5d	418	382	429	367	331	310	338	297	337
7d	525	577	585	548	486	473	482	479	505
10d	507	387	512	487	444	333	306	352	298
12d	356	380	374	280	368	362	416	265	219
14d	377	279	478	347	363	352	290	285	336
17d	364	429	464	-	362	382	271	377	290
19d	381	447	414	417	311	378	231	379	279
21d	258	374	494	424	314	396	232	347	262
25d	368	414	476	510	400	341	351	323	361

Thrombocyte counts x 10^3 per cu. mm. of piglets in third litter ex 306 by 311.

Table 12 Antiglobulin consumption test. Serum ex 306; platelets ex piglets in third litter ex 306 by 311.

	Reciprocal dilutions of APG									
	8	16	32	64	128	256	512	1024	2048	4096
APG control	++	++	++	++	++	++	++	++	(+)	-
Platelets ex 563	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	+	++	++	++	++	++	(+)	-	-	-
Platelets ex 564	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	+(+)	(+)	-	-	-	-	-
Platelets ex 565	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	++	+	-	-	-	-	-
Platelets ex 566	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	++	(+)	-	-
Platelets ex 567	++	++	++	++	++	++	++	(+)	-	-
+ PI serum	++	++	++	++	++	++	(+)	-	-	-
+ HI serum	++	++	++	++	++	++	+(+)	-	-	-
Platelets ex 568	++	++	++	++	++	++	++	+(+)	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	++	++	+	-	-	-
Platelets ex 569	++	++	++	++	++	++	+(+)	-	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	+(+)	(+)	-	-	-	-	-
Platelets ex 570	++	++	++	++	++	++	++	+	-	-
+ PI serum	++	++	++	++	++	++	+(+)	-	-	-
+ HI serum	++	++	++	++	+	-	-	-	-	-
Platelets ex 571	++	++	++	++	++	++	+(+)	(+)	-	-
+ PI serum	++	++	++	++	++	++	+	-	-	-
+ HI serum	++	++	++	+	-	-	-	-	-	-

APPENDIX VII

Reprinted from THE VETERINARY RECORD, August 24th, 1968. Pp. 203-4.

Iso-immunisation in the Parturient Sow by Foetal Red Cells

Sir,—The "natural" antibody, anti-A, is frequently found in the serum of pigs whose red cells are A negative. Other naturally occurring red cell antibodies in pigs were reported by Szent-Iványi and Szabó (1954) who, when studying the pathogenesis of haemolytic disease of newborn piglets reported finding three other such antibodies as well as anti-A. This work has never been confirmed and supplies of these pig serum samples are no longer available for examination and classification into the present day nomenclature for blood group systems in pigs. Goodwin, Saison and Coombs (1955) studied the iso-antibodies produced in pigs injected with crystal violet swine fever vaccine. However, they did not preclude the possibility of naturally occurring haemolytic disease in pigs by transplacental immunisation and found red cell antibodies other than anti-A at low titres in a small number of unvaccinated pigs.

In the present investigation a sow in a minimal disease pig unit was found to have low serum titres to red cells from an A negative pig. Using red cells from animals of known red cell type, absorptions were carried out on the serum samples of this sow and it was found that the main factor to which antibody had been produced was Ea. The question now arose as to how this anti-Ea had been produced. A complete history of the animal was available and this precluded the possibility of previous injection with red blood cells. It is known, however, that the sow had been mated to an Ea positive boar at a previous pregnancy and had given birth to Ea positive piglets. The possibility existed therefore of immunisation of the sow by Ea positive red cells of the foetuses passing into the maternal circulation during pregnancy or at parturition. At the time of sampling the sow was in pig to an Ea negative boar. Titres were followed throughout pregnancy and after parturition, and no variation was observed, which was to be expected as all the piglets were Ea negative. After weaning, the sow was artificially inseminated with semen from an Ea positive boar. By means of the direct tube agglutination, haemolytic and indirect sensitisation (antiglobulin) tests, serum antibody titres in the sow to red cells from four selected animals (A +ve Ea +ve; A +ve Ea −ve; A −ve Ea +ve; and A −ve Ea −ve) as well as from the boar (A −ve Ea +ve) used for the insemination, were followed. For carrying out the tests, red cells from these animals, collected in acid citrate dextrose, were washed four times and suspended in phosphate buffered saline at *p*H 7.2.

Blood samples from the sow were collected in dry Vacutainers* from a marginal auricular vein at fortnightly intervals during pregnancy, at parturition, at five and 10 days *post partum* and then at weekly intervals up to weaning. These were placed in an incubator at 37° C. for 30 minutes to promote clot formation and then left in a refrigerator at +4° C. overnight to allow for adsorption on to the red cells of any cold auto-agglutinating antibodies present in the serum. The blood was centrifuged and the serum decanted and inactivated in a water bath at 56° C. for half an hour before testing.

As well as titre, the intensity of reaction was recorded using a scale 0 to 5. In the direct agglutination test, when all cells were agglutinated, a score of 5 was recorded; 80 per cent. cells agglutinated, 4; 60 per cent. agglutinated, 3; 40 per cent. agglutinated, 2; 20 per cent. agglutinated, 1; and below this no score was recorded. Similarly with the direct sensitisation test a score system of 0 to 5 was used to record reactions. It must be noted that the scores for the two types of test are not strictly comparable.

Throughout the period of study no antibodies were found to the red cells of the A −ve Ea −ve animal. Titres to the A +ve Ea −ve animal remained more or less constant at 1 in 2, giving a score of 5 or 6 by direct agglutination. As shown in Fig. 1, titres to the boar's red cells and to cells from the other A −ve Ea +ve animal remained fairly constant at 1 in 2 (score 6 or 7) throughout pregnancy right up to parturition. After this there was a sharp rise starting at five days *post partum* reaching a peak at 10 to 17 days *post partum* with a titre of 1 in 16 (score 19). This level was maintained for about a month when it began to tail off. The titres to the A +ve Ea +ve animal followed the same pattern but were slightly higher presumably due to the combined action of the anti-A present along with the anti-Ea. With the haemolytic test, anti-A only was detected, remaining more or less constant throughout the period of study.

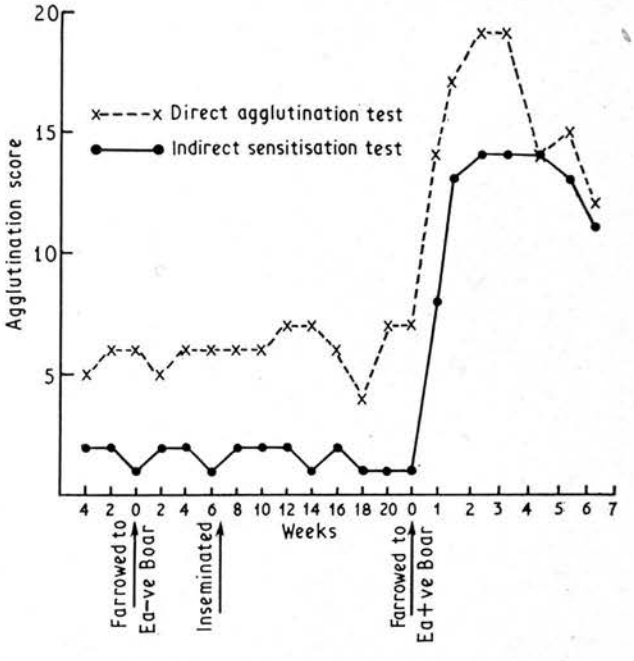

FIG. 1.—Antibody titres in sow's serum to red cells ex boar (A −ve Ea +ve) and another A −ve Ea +ve animal, showing increase in antibody level after giving birth to Ea +ve piglets.

* Becton, Dickenson and Company, Columbus, Nebraska.

To date, reports of haemolytic disease of newborn piglets have involved iso-immunisation of the sows by crystal violet vaccination for swine fever, Buxton, Brooksbank and Coombs (1955), Goodwin, Heard, Hayward and Roberts (1956), Böhm, Senk and Angus (1963), and most recently Mogi, Hosoda and Himeno, (1966) and Himeno, Nagano, Mori, Mogi and Hosoda (1967)—or by the direct injection of whole blood from the sire of the piglets into the sow before farrowing—Bruner, Brown, Hull and Kinkaid (1949), Andresen and Baker (1963). In no cases so far reported can the direct injection of erythrocytes into the sow be excluded. The findings presented support the hypothesis that immunisation of the sow by the foetal red cells can occur at parturition. Ea was the main factor involved in this case. With repeated matings to an Ea positive boar there is the possibility that future litters from this sow could be affected by haemolytic disease of the newborn.

I am indebted to the Pig Industry Development Authority for financial aid, to Professor A. Robertson for facilities provided within his department and in the minimal disease piggery, and to Dr. P. Imlah for his generous advice and guidance.

June 26th, 1968.

Yours faithfully,

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The Occurrence of Red cell Iso-antibodies in the Sera of Dams of Piglets Showing Thrombocytopenic Purpura

Sir,—Thrombocytopenic purpura in piglets was first reported in Norway by Stormorken, *et al.* (1963) and in the U.K. by Saunders *et al.* (1966). These have been followed by further descriptions of the clinical conditions, (Lie, 1968; Saunders & Kinch, 1968). It is considered by these workers that the thrombocytopenia is due to iso-immunisation of the sow by incompatible foetal thrombocytes. Antibodies so produced are absorbed by the piglets from the colostrum to produce thrombocytopenia in those piglets whose thrombocytes are incompatible. Due to the lack of a reliable serological test for thrombocyte antibodies in pigs, it has not yet been possible to demonstrate a rise in titre of thrombocyte antibodies in the sera of the dams of these litters either during gestation or after parturition.

However, Linklater (1968) has shown that iso-immunisation of the sow by foetal red cells can occur at parturition. Therefore, it was decided to look at the sera of the dams of thrombocytopenic litters, to see if red cell antibodies had been produced.

Serum samples from the dams of clinical cases were received from the V.I. centres at Cambridge, Aberdeen and Edinburgh. Tests for red cell antibodies were carried out using the direct agglutination, haemolytic and indirect sensitisation (antiglobulin) techniques. Doubling dilutions of serum were tested against red cells from five animals of known red cell type in order to cover various combinations of all known types in our blood group panel. With our own typing and the co-operation of J. Hojny* this panel has now been extended to cover 54 known red cell factors. Where antibodies were demonstrated absorptions were carried out to determine against which red cell factors they had been produced.

Of 24 samples examined, 12 were negative for iso-antibodies, but seven of these contained the natural antibody, anti A alone. The other 12 showed evidence of red cell iso-antibodies, six of which had anti A as well (Table I). Of the iso-antibodies present, anti Ea was the most common (Table II) and in some sera more than one iso-antibody was found. Vaccination with crystal violet swine fever vaccine can be precluded in all but two of these animals, one of which had a red cell iso-antibody present in its serum, the the other of which had not.

TABLE I

Serum examination	No. of animals
No antibody	5
Anti A alone	7
Anti A and iso-antibody	6
Iso- antibody alone	6
Total	24

TABL II

Iso-antibody	No. of times isolated	Titre range
anti Ea	5	4—16
anti Eb	2	4—32
anti Ed	1	8
anti Ee	1	2
anti Ka	1	1
anti Kb	2	1—2
anti M system	1	1
unidentifiable	2	1
Total	15	

These results support the hypothesis that iso-immunisation by foetal cells is occurring in sows which have produced thrombocytopenic litters. That red cell iso-antibodies are not produced in all cases may merely be due to the fact that, while the thrombocytes of the foetuses may be incompatible with the dam in all cases, the foetal red cells are not necessarily always incompatible with the dam.

I wish to thank the Meat and Livestock Commission for financial support while carrying out this investigation, Dr. P. Imlah for his advice and guidance, and Messrs. C. N. Saunders, P. L. Shanks and Dr. J. A. A. Watt for the supply of clinical cases.

March 10th, 1970. Yours faithfully,

K. A. LINKLATER.

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