STUDIES ON NORMAL ANTIBODIES

FOR SWINE ERYTHROCYTES

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
University

By

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1956

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ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. T. M. Ludwick for inspiring guidance and help during all phases of this problem. His patience, thoughtfulness, and timely suggestions are especially appreciated.

For technical advice and many patient explanations, the author is especially grateful to Dr. E. J. Lazear. Without his help and council, this work would not have been possible.

For providing facilities and equipment for this study, the author wishes to thank Dr. Fordyce Ely of The Ohio State University and Superintendent R. E. Hodgson of the Southern Experimental Station, University of Minnesota.

Taking blood samples would not have been possible without considerable help. For teaching the bleeding technique, the author is indebted to Dr. L. C. Ferguson. For assisting with the actual bleeding, special thanks are due to Dr. L. E. Kunkle and his colleagues of The Ohio State University Meats Laboratory and to Mr. George Sutter and Mr. Elmer Minske of the Southern Experimental Station.

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INTRODUCTION

Since 1900, when Landsteiner discovered differences in human bloods, man has tried to learn more about these normally occuring genetic expressions. Typically, an antigen is a substance that, when introduced into the body, will stimulate the production of antibodies against that antigen. This physiological reaction is commonly stimulated to bring about immunity against a specific disease. These antibodies are usually referred to as "immune antibodies". "Normal antigens" and some of the corresponding antibodies are not foreign materials or the result of introducing foreign materials into the body. These are factors in pathology only when their presence will result in an undesirable antigen-antibody reaction such as "transfusion accidents".

During the past fifty-five years, our knowledge of normal antigens and antibodies has increased very greatly. This knowledge has been put to many and varied uses. Most research, until recent years, has been with humans; but many papers, dealing with other species, have appeared in the last twenty years.

At the present time, the mode of inheritance is known for many antigens found in several species. This knowledge has been put to many practical uses. One of the most fascinating uses is the study of species relationships through similarities in immunological reactions.

Some of the more practical applications of immunogenetics are illustrated in "transfusion accidents" and erythroblastosis fetalis in humans. When blood transfusions were first used, recipients frequently suffered serious intravascular reactions which often resulted in death. Now, by typing the blood of the donor and recipient for both the classic ABO and Rh systems, transfusion accidents rarely occur. Also, "Rh babies" have an excellent chance of survival since the causative antigen-antibody raaction is known and techniques for preventing dangerous cell agglutination have been developed.

Blood typing cannot identify an individual human or lower animal except to place that individual in a particular blood group. Nevertheless, by typing and exclusion, serological studies of normal blood factors are useful to jurisprudence. Criminologists utilize extensively blood grouping techniques to make their work more efficient. Parentage determination for humans, and especially for cattle, is now more positive because blood grouping can definitely exclude certain individuals.

Geneticists and animal breeders have long been looking for "marker genes" to improve the speed and accuracy of genetic evaluation. When blood factor genes are found linked to genes of more practical importance, a new and valuable tool will be available for inheritance studies and animal improvement. No doubt, immunogenetics will grow rapidly and increase in importance in the next few years.

Cattle perhaps have been studied more extensively than any of the other lower animals. Most of the common domestic and some laboratory animals have been studied in considerable detail. Some normal

antigens and antibodies have been identified and the mechanics of inheritance determined for these species. Yet, one of our most common and economically important species has been studied only sparingly. The literature is almost void of any blood grouping attempts in swine. It is desirable that our knowledge of this species be brought up to a level comparable to that of other important animals. This information would have, no doubt, useful application for swine.

REVIEW OF LITERATURE

Raffel (41) defines an antigen as "a substance which upon introduction into the animal body causes a response, which is revealed by the ability of the body fluids to react with the provoking substance"; and an antibody as "a humoral globulin produced by the body in response to an antigen, and capable of reacting with the antigen in some observable way". The latter definition is for immune antibodies; however, normal antibodies are specific globulins characterized by influences other than a foreign provoking substance.

Landsteiner's discovery in 1900 (21) of antigenic differences in man's blood cells opened a new field of study. Many workers have found additional antigens and antibodies in humans and many other species. By 1913, papers were published (10, 13, 47, 48, 49) identifying additional antigens and antibodies, and attempts were made to explain their mode of inheritance even though the science of genetics was also in its infancy. Since that time, most species of domestic animals have been studied extensively. Swine, however, have been studied very little.

The isoagglutinins of the classic ABO system, worked out on the basis of Landsteiner's original work, are the only examples of normally occuring red cell antibodies in humans. Additional antigens have been discovered, but only anti-A and anti-B can be classed as normal antibodies. Other important antigens are M and N identified by Landsteiner and Levine (25, 26) and the Rh factors discovered by

Landsteiner and Weiner (28). The generally accepted explanation of the inheritance of human blood groups was made by Bernstein (1). Ottenberg (37) gave one of the first explanations of "transfusion accidents", and the discovery of the Rh factors explained erythroblastosis fetalis in babies. Raffel (41) points out that blood cell isoantigens are of practical concern in (1) blood transfusions, (2) hemolytic disease of newborn, and (3) parentage determination.

The early workers soon began to study other animals in an attempt to learn more about antigens and antibodies. Todd (47), and White and Todd (48), using a hemolytic test with guinea pig complement, found that 75 per cent of cattle tested possessed isolysin. These workers also studied goats, sheep, and rabbits and employed injection and absorption techniques to further their studies. Fishbein (10), using the agglutination technique, found evidence of isoagglutinins in swine, cattle, sheep, rabbits, and dogs, but not frogs. Isoagglutinins were also observed in steers and rabbits by Ottenberg and Friedman (38). Three blood groups in cattle were suggested by Little (31).

While these early workers found antigenic differences in animals, little was done for over a quarter of a century to study individual antigens and antibodies. In 1941, Ferguson (7) identified nine antigens in cattle, and suggested their mode of inheritance. Additional antigens in cattle were suggested by Ferguson et al. (9) in 1942. Anti-J, which is probably the only normal blood cell antibody in cattle, has been studied extensively. The similarities between cattle

J, human A, and R of sheep were investigated by Sorenson and co-workers (44). They found that these three antibodies were very much alike in their serological reactions. Elliott and Ferguson (6) determined the frequency of normal anti-J and the production of immune anti-J. Factors affecting variations in titer of anti-J were presented by Stone (45). He observed lower titers during the winter and spring and at or near parturition. In view of the marked individual variations in titer, Stone doubted the existence of cattle without either J or anti-J.

An excellent review of the literature dealing with blood groups of animals is presented by Ferguson (8). This paper covers blood groups in eight species or groups of species.

The genetic aspects of human, dove, and cattle antigens have been reviewed by Irwin (15). Inheritance is usually controlled by simple mendelian factors according to Irwin. However, there are probably two alleles for human A, while Rh is probably controlled by genes at three loci with at least two alleles at each loci. Antigens in the B system in cattle appear to be controlled by a complex gene make-up rather than by a simple mendelian pair of factors.

Ycas (52) found nine cellular antigens and two normally occuring isoantibodies in sheep. She used the hemolytic test, and sheep and rabbit sera containing antibodies developed by injecting specific sheep cells into these two species. No evidence of anti-R was found in lambs at birth but soon after the lambs ingested colastrum this antibody was detected.

Several species of fowl have been studied extensively. One of the first demonstrations of antigenic differences in chickens and ducks was by Landsteiner and Miller (27). Irwin and co-workers (15, 16, 17) have studied doves and pigeons. They found certain antigens in only one species (species specific) and other antigens common to several species. In some crosses, they observed a specific antibody not found in either parental species. These workers refer to this antibody as a "hybrid antibody" which results from the interaction of two different sets of genes. McGibbon (32) studied six antigens in ducks. He also found species specific as well as common antigens and evidence of a "hybrid antibody".

Agglutination of red blood cells was observed in ear veins of transfused rabbits by Rous and Robertson (42). Levine and Landsteiner (30) identified an isohemagglutinogen in transfused rabbits. They suggested several isoagglutinins but did not identify them. Knapfmacher (19) suggested a third isohemagglutinogen that he called H_6. These are apparently inherited as simple mendelian units. The animals homozygous for the antigen could be differentiated from the heterozygous animals by titer differences according to Knapfmacher.

One of the first papers suggesting in vivo antigen-antibody reactions in dogs was by Ottenberg et al. (40). Anemia in injected dogs was observed by Melnick and co-workers (33). They suggested two blood groups. A similar observation was made by Wright (51) and by Young et al. (53). The latter workers point out that four isohemagglutinins are known in dogs. They effected passive transfer through the milk to

susceptible puppies and observed retarded growth and hemolytic complications after nursing. Ingebrigtsen (13) and Ottenberg and Thalhimer (39) found evidence of isoagglutinins in cats as well as dogs.

A pathological condition is foals has been traced to an antigen-antibody reaction (5). This condition was termed "neonatal isoerythrolysis" by Hagen and Bruner (11) and results in intravascular lysis of foals' red blood cells after nursing.

Evidence for the presence of isoagglutinins in swine was presented by Fishbein (10) in 1913. Serum from only two of sixty hogs failed to react with any cells, but he could not demonstrate any grouping relationship. Szymanowski (46) showed that pigs could be separated into three groups: those possessing antigen, those possessing antibody, and those devoid of both antigen and antibody. A more complex blood group structure was shown by Harte (12). His observations could only be explained on the basis of at least two antigen-antibody systems.

Kuhns (20, 21) suggested four blood factors in swine which he called Pi_1 - Pi_4. Ferguson's review (8) shows that very little basic work has been done on normal antibodies for red blood cell antigens in swine.

The similarity of human A to a factor found in swine was reported by Landsteiner and Chase (23). This substance, found in the gastric mucin of swine, was shown to be carbohydrate in nature (23, 24).

A hemolytic disease of newborn pigs was demonstrated experimentally by Bruner et al. (2), and observed to occur naturally in several litters by Kershaw (18). In both instances the pigs observed were

normal (by gross observation and hemoglobin analysis) at birth, but showed definite anemic symptoms soon after nursing, followed by death within 48 hours for almost all pigs. The former workers found that the pigs' blood matched the sire's, but not the dam's, and the dam's milk had a very high titer of antibodies against the sire's cells. Although this general type of phenomenon has been observed in several species (2, 5, 28), Chavez (4) was unable to demonstrate experimentally any evidence of hemolytic disturbance in calves, nor could any reference be found in the literature for such a condition in cattle.

The literature contains no references to linkage between genes for antigens or antibodies and physical or physiological characteristics of swine. This is understandable since so little is known about swine blood groups. Elliptocytosis genes have been linked with the Rh genes in humans (3), and Nair (36) found evidence for linkage of the factors for fat percentage in cattle with the genes for the B antigen system.

MATERIALS AND METHODS

Animals

During the preliminary phase of this study, blood samples were taken, at the time of slaughter, from 104 pigs in The Ohio State University Meats Laboratory. These pigs represented several different breeds and crosses. In many cases, the breed was not known.

All other hogs used in this study were from The University of Minnesota Swine Breeding project. Most of these hogs were at the Southern Experiment Station and consisted of mostly Minnesota No. 1 and Minnesota No. 2 crosses; although two Poland China lines and several other lines were also represented. Seventy-one Minnesota No. 1's at the North Central Station were bled and several Minnesota No. 2's and crossbreds at the Rosemount Research Center were also bled.

Practical consideration of availability and convenience usually determined which pigs were to be bled; although all animals used for breeding purposes at the North Central and Southern Stations were included. More females than males were tested since more females were used for breeding purposes. Several animals were bled two or three times to study titer variations at different ages and under different physiological conditions.

Antigens and Antibody Determination

Bleeding was done from an ear vein or with syringe and needle from the jugular vein. Two samples were taken, one into isotonic sodium citrate and the other into glass or plastic tubes. The citrated

sample was used to provide cells and the other sample was allowed to clot and the serum removed. The serum was stored at -12° C and the cells at +4 to 6° C until used.

A hemolytic test modelled after the one of Ferguson (7) was used. Fresh rabbit serum was found to react slightly with pig cells; therefore, this serum was absorbed at 0°C with 10 per cent, washed pig cells before use as a source of complement.

The test consisted of adding 0.05cc. of 3 per cent washed cells to serological tubes containing 0.1cc. of diluted serum. Most tests were made with serum diluted 1:2 with physiological saline. After incubating the cells and serum for 30 minutes, 0.05cc. of undiluted absorbed rabbit serum was added to each tube. Three readings were made at 45 minutes, 1 hour and 45 minutes, and 2 hours and 45 minutes, after adding the complement. Eight degrees of lysis were recorded from 0 to 4+. A 4+ value was given when lysis was complete.

Most bloods were tested with 20 to 30 different sera, a few of which were of known activity. All sera were tested with at least 20 different bloods and many were tested against 50 or more. Serum may be stored in the frozen state for long periods of time; therefore, most sera were tested with a large number of blood cells. The cells rarely remained intact for more than 10 days, even when stored at +4 to +6° C.

To facilitate reference, the terms positive (or active) and negative (or inactive) cells and sera are used. A positive anti-A serum is one that, at a 1:2 dilution, will lyse at least 50 per cent of the

cells, and a positive anti-B serum will lyse at least 10 per cent of the cells. Positive cells are at least 50 per cent or 10 per cent lysed by positive anti-A and anti-B sera respectively.

Soluble antigens were detected by inhibition techniques as outlined by Lazear (29). In this test, 0.05cc. of serum to be tested was incubated for 30 minutes with 0.05cc. of known positive serum. Then, 0.05cc. of 3 per cent suspension of known positive cells were added and incubated for 30 minutes. The tubes were then centrifuged and the liquid aspirated by means of a water pump. The original volume was restored with physiological saline, complement added, and read as a regular hemolytic test. In this test, if soluble antigen were present, it would unite with the specific antibody present and inhibit this antibody from sensitizing the positive cells; therefore, the presence of soluble antigen inhibits lysis. If, however, no soluble antigen were present, antibody would be available to sensitize the cells and lysis would occur when complement was added. A zero (0) reading indicates that soluble antigen was present.

RESULTS AND DISCUSSION

The blood from over six hundred hogs was crossmatched using the hemolytic technique. The data obtained were studied in several different ways in an attempt to demonstrate the presence and characteristics of swine antigens and antibodies. The original data for this study are on file at the Southern Experiment Station, University of Minnesota, Waseca, Minnesota. The results of each category studied will be presented separately.

Identification of Antigens and Antibodies

In this study, the various bloods were identified according to the pig from which the blood was taken. For example, cells L37 and serum L434 were the blood cells and blood serum from pigs L37 and L434 respectively.

During the early phases of this study, all of the sera could be placed in one of two groups. One of these groups consisted of the sera which contained antibody. The antibody containing sera were those capable of bringing about hemolysis of some bloods. The sera in the other group (or group two) consisted of those bloods which did not have demonstrable antibody.

The sera in group one were characterized by the fact that all of them reacted with the same bloods. The level of antibody or the degree of reactivity within this group was quite variable; however, all of the sera did give some demonstrable reaction with the same blood cells.

Conversely, the blood cells could also be placed in two groups; namely, those which were lysed by group one sera and those which were not lysed by the same sera. Again the degree of reactivity was quite variable. Some cells were completely lysed while others showed a very slight amount of lysis. It was interesting to note that the more active cells with one sera were also more active with all sera, while the less active cells with one sera were also less active with all sera.

Later it was observed that some sera did not follow the same pattern of activity as those sera in group one described above. These sera (group three) were characterized by the fact that all would react with a different group of cells than those which reacted with group one sera. The level of antibody in the group three sera was quite variable; however, all sera of this group gave the same demonstrable reaction with the same blood cells. The amount of hemolysis caused by group three sera was usually less than that brought about by group one sera. A fourth group of sera consisted of those which did not cause lysis of the same bloods as did group three sera.

The blood cells could also be placed in two groups regarding their reactivity with group three sera; namely, those that were active and those that were not. Again, the degree of activity was quite variable. Some cells were lysed very slightly by group three sera and some were completely hemolysed.

Occasionally the blood from one pig was lysed by both group one and group three sera, indicating the presence of both antigens in

that pig's blood cells. Also some sera would lyse blood cells of both groups which would indicate that both antibodies were present in that sera. In no case did the sera from a pig bring about lysis of the blood cells from that pig.

Typical examples of the different groups of cells and sera are shown in Table 1. Sera of the first group, including O-49, K774, L183, and L372, lysed cells such as L37, L155, L429, and L434. These sera did not lyse cells K774, L175, L372, and L680. However, sera L305, L323, L429, and L434 would lyse the latter group of cells, but not the former group. Some cells, such as L229, L250, L315, and L531, were lysed by both groups of sera, but serum from these animals were completely inactive.

To facilitate reference, serum O-49 and all sera that reacted in the same manner are designated anti-A, or a, and cells lysed by these sera are A cells and have A antigen. Cells lysed by L434 and like sera have B antigen and L434 serum has anti-B, or b, antibody.

These two distinct groups of reactions indicated that two different antigen-antibody systems are present in swine. These are designated the A and B systems corresponding to the antigens and antibodies described above. The consistent specificity shown by cells and sera indicate that the factors present are probably antigens and antibodies and not gross non-specific substances. Specific antibodies could be removed by absorbing with the corresponding cell antigens.

TABLE 1

Identification of A and B Antigen-Antibody Systems
(Reaction of Cells and Sera from various animals)

Cell Groups	Anti A Sera				Anti B Sera				Anti A and B Sera			
	O-49	K774	L183	L372	L305	L323	L429	L434	L133	L291	L385	L395
A Cells												
L37	+	+	+	+	0	0	0	0	+	+	+	+
L155	+	+	+	+	0	0	0	0	+	+	+	+
L429	+	+	+	+	0	0	0	0	+	+	+	+
L434	+	+	+	+	0	0	0	0	+	+	+	+
B Cells												
K774	0	0	0	0	+	+	+	+	+	+	+	+
L175	0	0	0	0	+	+	+	+	+	+	+	+
L372	0	0	0	0	+	+	+	+	+	+	+	+
L680	0	0	0	0	+	+	+	+	+	+	+	+
A and B Cells												
L229	+	+	+	+	+	+	+	+	+	+	+	+
L250	+	+	+	+	+	+	+	+	+	+	+	+
L315	+	+	+	+	+	+	+	+	+	+	+	+
L531	+	+	+	+	+	+	+	+	+	+	+	+
Neither A nor B Cells												
L133	0	0	0	0	0	0	0	0	0	0	0	0
L291	0	0	0	0	0	0	0	0	0	0	0	0
L385	0	0	0	0	0	0	0	0	0	0	0	0
L395	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2

The Presence of Soluble Antigen A
in 126 Pig Sera Negative for Anti-A

Number with both Cellular & Soluble A	Number with Soluble Only	Number with Cellular Only	Number with Neither Cellular or Soluble
95	13	6	12

The presence of soluble antigen was determined in the sera of 126 pigs in which anti-A was absent. Table 2 shows the results of these determinations. Both cellular and soluble antigen were found in the blood of 95 pigs; 13 showed soluble only, 6 showed cellular A only, and 12 showed neither form of A. No definite evidence of soluble B could be found in 220 pigs tested.

In designating each pig's blood type, several different combinations may be found. A pig may have cellular and/or soluble A, or anti-A, or neither antigen or antibody of the A system. In addition, a pig may have B or anti-B or neither of the B system.

Kuhn's finding (20, 21) of four antigen-antibody systems in swine was not confirmed by this study. The two systems found are the same number reported by Harte (12) for swine and the same number found in humans (41) and sheep (52). Cattle probably have only one normally occurring antibody, anti-J (44). Cattle, humans, and sheep do have additional normal cell antigens.

Incidence of Antigens and Antibodies

Tables 3 and 4 give the incidence of antigens A and B, and anti-A

and anti-B by breed groups. Of 573 pigs tested for the A system, with definite results, 146 (25.5 per cent) possessed A, 410 (71.5 per cent) had anti-A and 17 (3.0 per cent) did not react with either antigen or antibody. For the B system, 431 gave definite results. Two hundred fifty-one (58.2 per cent) had B, 139 (32.3 per cent) showed presence of anti-B and 41 (9.5 per cent) did not react either way.

It is doubtful whether any pigs are devoid of both antigen and antibodies. The wide variation in titer indicates that, at the time of some samplings, the titer may have been too low to detect the presence of antibody; even though the animal had antibody. This could account for the 3 per cent that showed no evidence of A or Anti-A. The presence of either antigen or antibody in all animals agrees with Stone's suggestion (45) that all cattle possess either J or anti-J and Raffel's opinion (41) that all humans have either A or anti-A. Variations in titer may account for the 9.5 per cent of the pigs with neither B or anti-B. The titer of anti-B was usually low and a slight drop in titer would make it difficult or impossible to detect. Therefore, it is suggested that all pigs possess B or anti-B. It is not likely that antigen was missed in testing because all cells were tested against known positive sera with a relatively high titer.

The over-all incidence of antigens and antibodies, observed in this study, is not in agreement with that reported by Kuhns (21) or Harte (12). This is understandable because each study was based on

TABLE 3

Incidence of A and Anti-A
by Breed Group[a]

Sire	Dam	Number Tested	Number Animals Reacting Positively		
			A	Anti-A	Neither
M-PC	M-PC	26	0	26	0
1	1	69	12[b]	57	0
1	C-PC x 2	24	10[c]	14	0
1	3 x 2	18	3[c]	14	1
1	SP- x 2	34	8	22	4
1	B_2 x 2	34	1	32	1
B_2	SP	14	11	3	0
2	1 x 2	46	2	44	0
Mixed		205	49	153	3
Other and Unknown		103	50[d]	45	8
Total		573	146	410	17
Per cent			25.5	71.5	3.0

[a] 1, 2, and 3 refer to Minnesota No. 1, Minnesota No. 2, and Minnesota No. 3 respectively. M-PC and M-PC are two different Poland China lines. SP and B_2 refer to San Pierre and Beltsville No. 2 respectively.

[b] Includes 8 positive for soluble A only.

[c] Includes 1 positive for soluble A only.

[d] Includes 3 positive for soluble A only.

TABLE 4

Incidence of B and Anti-B
by Breed Group[a]

Sire	Dam	Number Tested	Number Animals Reacting Positively		
			B	Anti-B	Neither
M-PC	M-PC	26	14	11	1
1	1	64	63	0	1
1	C-PB x 2	13	10	3	0
1	3 x 2	16	8	7	1
1	SP x 2	28	9	12	7
1	B_2 x 2	31	15	14	2
B_2	SP	5	0	1	4
2	1 x 2	43	35	3	5
Mixed		202	97	85	20
Other and Unknown		3	0	3	0
Total		431	251	139	41
Per Cent			58.2	32.3	9.5

[a] 1, 2, and 3 refer to Minnesota No. 1, Minnesota No. 2, and Minnesota No. 3 respectively. M-PC and C-PC refer to two different Poland China lines. SP and B_2 refer to San Pierre and Beltsville No. 2 respectively.

a restricted group of pigs that were, likely, not of similar genetic backgrounds. It is possible that the A system referred to in this study is analagous to Pi_1 reported by Kuhns (21), but the variation in frequencies of antigen and antibody is too wide in the two studies to prove the similarities.

It is evident that antigen A may appear in cellular or soluble form. These two forms tend to appear together; however, the fact that both forms were found independent of the other indicates that separate determining factors control the presence of these two forms. The 12 showing neither antigen or antibody may actually possess antibody in too low a titer to be detected in these tests.

None of the 64 Minnesota No. 1 pigs, tested for the B system, had anti-B, but 63 of the 64 showed B antigen. The incidence of cellular A for Minnesota No. 1 pigs was only 4 of 69 tested, although 8 others possessed soluble A. The two Poland China lines apparently differ in their blood types. None of the 26 M line Poland China possessed A, but all had anti-A. Fourteen of the 26 reacted as B and 11 showed presence of anti-B. No pure C line pigs were tested, but crosses with Minnesota No. 1 and Minnesota No. 2 showed 10 of 24 with A, 14 with anti-A, and 10 of 13 with B, and only 3 with anti-B. The Minnesota No. 2 shows a low incidence of A since 1 x 2 crosses showed only 2 of 46 with A and 44 with anti-A. These crosses showed 35 of 43 with B and only 3 with anti-B.

The Beltsville No. 2 and San Pierre evidently possess a rather high incidence of A as shown by the rather high incidence of this

antigen in crosses involving these breeds. These breeds also appear to have a higher incidence of anti-B than the Minnesota 1 and 2 breeds.

Many breeds were not represented in this study; therefore, an over-all incidence for swine cannot be suggested. The mixed group would perhaps be the nearest to the general population because many breeds and combinations of breeds are represented in this group. The ratio of anti-A to A in this group was about 3:1 (153 to 49). Too few were tested for the B system to draw any conclusions.

Sex Differences in Incidence of Antigen and Antibody

To make a comparison of the incidence of antigen and antibody by sex, a group of related males and femaled was selected. Littermate boars and gilts were selected for this comparison. All litters in which both males and females had been tested and all pigs tested in these litters are included. The number of males and females in each litter is not necessarily equal.

These results gave 98 males and 138 females tested for the A system, and 91 males and 132 females tested for the B system. Twenty-three (23.5 per cent) of the males and 32 (23.2 per cent) of the females possessed A. Anti-A was found present in 74 (75.5 per cent) of males and 106 (76.8 per cent) of females. Of the 91 boars tested for B, 48 (52.7 per cent) were positive compared to 68 (51.5 per cent) of females. Thirty-five boars (38.5 per cent) and 51 (38.6 per cent) of females were Anti-B positive. A Chi square test for significance of difference showed no differences in incidence between sexes.

TABLE 5

Incidence of Antigens A and B
and Anti-A and Anti-B by Sex

	Males			Females		
	No. Tested	No. Positive	Per Cent Positive	No. Tested	No. Positive	Per Cent Positive
Antigen A	98	23	23.5	138	32	23.2
Anti-A	98	74	75.5	138	106	76.8
Neither A or Anti-A	98	1	1.0	138	0	0
Antigen B	91	48	52.7	132	68	51.7
Anti-B	91	35	38.5	132	51	38.6
Neither B or Anti-B	91	8	8.8	132	13	9.9

Sex differences in incidence of antibody have not been found in other species; therefore, the results reported here are not unexpected. Sex apparently has no influence on the incidence of antigens or antibodies.

Variation in Titer of Antibody

The antibody titer of a serum sample is the average of all reactions from 0± to 4+ at a 1:2 dilution. Since the titer for most sera was very low this method of designating titer is used. To expedite averaging, numbers 1 - 7 were assigned to 0±, 0+, trace, 1+, 2+, 3+, and 4+ respectively. The age or time designation of a sample is based on the date the sample was drawn regardless of the date tested.

Average titers include all animals bled within that category, which showed any definite reaction. Most pigs are represented only once, but several appearing more often were re-bled to obtain more information relative to titer variation.

Anti-A was first found in a 22-day old pig. Three other pigs bled between the third and fourth week of age also showed Anti-A. Eleven pigs bled earlier than three weeks failed to show any Anti-A. From three to four weeks of age, the titer rose to an average of 1+ at about eight weeks of age. From eight weeks to about five months, the titer remained at 1+ or slightly above when it again increased to an average of more than 2+ at seven to ten months of age. Thereafter, physiological disturbances of gestation and lactation alter the strength of reaction.

Anti-B followed a somewhat different pattern. This antibody was

TABLE 6

Age Differences In Antibody Titer
Lactating Sows Not Included

Age	No. Tested for Anti-A	No. + for Anti-A	Ave. Titer	No. Tested for Anti-B	No. + for Anti-B	Ave. Titer
Less 1 wk.	1	0	-	1	0	-
1-2 wks.	8	0	-	8	6	1.1
2-3 wks.	2	0	-	2	0	-
3-4 wks.	4	4	2.7	4	2	2.3
4-5 wks.	7	7	2.7	7	2	2.3
5-6 wks.	12	12	3.4	12	10	2.5
6-8 wks.	7	7	4.1	7	5	2.7
8-10 wks.	26	22	4.2	26	9	2.1
10-12 wks.	39	33	4.4	39	8	2.0
3-4 mo.	39	32	4.3	39	18	1.8
4-5 mo.	188	132	4.2	176	77	2.7
5-6 mo.	94	75	5.0	92	31	1.9
6-7 mo.	30	26	5.3	25	10	1.7
Over 7 mo.	16	14	5.7	16	8	2.0

observed in 6 of 8 pigs bled during the second week of age. Beginning at about three weeks of age, the titer increased to a maximum average of almost a trace at about eight weeks. Thereafter, the level fluctuated to some extent without any definite change. After the third week, the average titer of Anti-A was always higher than Anti-B.

The source of antibody at the younger ages is not known. For Anti-A, the antibody detected is likely the pig's own antibody. If this early antibody was from the colostrum, it should have been detected at earlier ages. The dams' blood types included Anti-A in most cases; therefore, Anti-A may have been passively transferred.

The six observations of Anti-B are likely colostrum antibody because all dams possessed Anti-B and it appeared at less than two weeks of age.

The seasonal variations are difficult to interpret because these variations are affected by age differences. The high titer during the fall months coincides with the high titer of six to nine months of age for the spring farrowed pigs. The winter titer was influenced by the higher titer of five to six month old pigs. The high titer during fall and winter averaged almost 2+ and reach a low of slightly less than 1+ during the summer months. This low titer was influenced by several post-lactating sows that had a lower average titer than pigs not influenced by lactation.

The seasonal variations observed in this study are much the same as the variations found for cattle Anti-J by Stone (45). He observed high titers in the fall, but the lowest titers occured in the spring.

TABLE 7

Seasonal Variations In Antibody Titer

Months	No. Tested for Anti-A	No. + for Anti-A	Ave. Titer	No. Tested for Anti-B	No. + for Anti-B	Average Titer
Sept. - Nov.	207	157	4.9	201	61	2.7
Dec. - Feb.	114	81	4.9	114	49	2.1
Mar. - May	76	65	4.3	76	29	1.8
June - Aug.	164	118	3.9	138	27	2.4

The greatest number of pigs tested in August, September, and October were born in March and April. The greatest number of pigs tested in November through February were born in August.

The effect of lactation on antibody titer is studied by comparing the serum titer of the same animals under different physiological conditions. Seventeen sows positive for Anti-A during the growing period were again studied during lactation and after weaning. For Anti-B, there were eleven sows. The pre-lactation titer is an average titer at four to eight months of age. Lactating sows were tested on the date of farrowing to 32 days past farrowing. The same sows were again tested 7 to 28 days after weaning.

The 17 sows positive for Anti-A showed an average titer of 4.7 (1.9 to 7.0) at four to eight months of age. The same sows averaged 3.4 (0 - 6.5) during lactation and 4.1 (1.0 - 6.5) after weaning. For Anti-B, the pre-lactation titer averaged 2.4 (1.0 - 4.0), dropped to 0.8 (0 - 2.5) during lactation and increased to 1.1 (0 - 3.0) after weaning. These titers are on the 1 - 7 scale with 1 being 0± and 7 being 4+.

The reduction in concentration of serum antibody found here is in partial agreement with Stone's findings for cattle Anti-J (45). His suggestion that antibody passing into the milk would account for the reduction in serum antibody. This is also a logical explanation for the reduction observed in this study. Stone found a reduced titer at or near parturition only, but a continued post-parturition reduction was observed in this study. It is possible that larger quantities of globulin pass into the sows' milk compared to cows' milk since the protein content of sows' milk is about twice that of cows. No reference in the literature was found stating the globulin level of sows' milk. Bruner et al. (2) found a very high titer of antibody in the sows'

TABLE 8

Effect of Lactation on Antibody Titer

	Average Titer During Growth 4-8 mo.	Average Titer During Lactation 0-32 days	Average Titer Post-Lactation 7-28 da.
17 sows Anti-A positive			
Range	1.9-7.0	0-6.5	1-6.5
Ave.	4.7	3.4	4.1
11 sows Anti-B positive			
Range	1.0-4.0	0-2.5	0-3.0
Ave.	2.4	0.8	1.1

colostrum but the titer later in lactation was not reported.

Similarity of Swine Anti-A to cattle Anti-J

The blood cells of 124 pigs were tested against Anti-A, Anti-B, and cattle anti-J. The results are shown in Table 9. Twenty of these cells were active with both Anti-A and Anti-J, but not with Anti-B. The cells from 3 pigs were active with all three antibodies, 47 were active against B only, and 54 were not active with any sera.

In every test, Anti-A reacted like Anti-J, except in magnitude of reaction. Anti-J usually reacted more strongly than Anti-A, but always with the same cells. Anti-A was not compared with other species; however, Sorenson (44) demonstrated similarities between cattle Anti-J, human Anti-A, and sheep Anti-R. It is, therefore, possible that swine Anti-A is similar to the above mentioned antibodies found in the latter two species.

Inheritance of Antigens and Antibodies

Table 10 lists the blood types of 62 pigs and the blood types of their parents. In all 62 pigs, the antigens and antibodies found were present in one or both parents, except one pig in which the dam may have had the antibody but it was not detected. Capital letters indicate presence of antigen, small case letters indicate presence of antibody, and o indicates that neither antigen or antibody was found.

Males of aB type mated to aB sows produced only aB offspring. The same boars with ab sows produced 5 aB and 1 ao offspring; and with Ao sows, 3 aB, 1 AB, and 1 Ab offspring were produced. Another pair of boars of ab type mated to ab sows produced only ab pigs, and when mated to aB sows, 9 ab, 4 aB, and 1 ao pigs were produced. When bred to Ab

TABLE 9

Similarity Of Swine Anti-A To Cattle Anti-J

(Reactions between various cells and sera)

No. of Animals	Cell Group	Serum Reagents Anti-A	Anti-B	Anti-J
20	A-	+	0	+
3	AB	+	+	+
47	-B	0	+	0
54	--	0	0	0

TABLE 10

Inheritance Of Antigen A and B,
Anti-A And Anti-B

Sire Blood Type	Dam Blood Type	Offspring Blood Type	Number
aB	aB	aB	16
aB	ab	aB	5
		ao	1
aB	Ao	aB	3
		AB	1
		Ab	1
aB	oo	aB	1
ab	aB	ab	9
		aB	4
		ao	1
ab	Ab	ab	5
		Ab	2
ab	Ao	ab	5
		Ab	3
		Ao	1
ab	ab	ab	4

A indicates presence of antigen A.
a indicates presence of Anti-A.
B indicates presence of antigen B.
b indicates presence of Anti-B.
o indicates the absence of any reaction for the antigen or antibody considered.

sows, 5 ab and 2 Ab pigs were farrowed; and with Ao sows, 5 ab, 3 Ab, and 1 Ao were produced.

The presence, in either or both parents, of antigens and antibodies present in the offspring, and differences in breed incidence demonstrates that the factors studied here are inherited. Non-specific substances, not under genetic control, would not be expected to react in a systematic manner or follow an inherited pattern. Therefore, it is evident that normal antigens and antibodies are present and their presence is controlled by genetic factors.

The numbers studied are too limited to enable an explanation of the mode of inheritance.

SUMMARY AND CONCLUSIONS

The bloods of approximately six hundred pigs were typed using techniques similar to those used with cattle. Data were obtained on the presence of antigens and antibodies and the effect of some environmental influences on the expression of these substances. On the basis of the analysis of these data, the following observations are presented:

1. Two normal antigen-antibody systems were found in swine. Antigens A and B, and antibodies Anti-A, or a, and Anti-B, or b, are the designations proposed for these two systems.

2. Anti-A was found approximately three times as frequently as antigen A but antigen B was found more often than Anti-B.

3. Antigen A was found in both cellular and soluble form.

4. Breed group differences in incidence were observed.

5. No sex differences in incidence were observed.

6. Age apparently affects titer. This is especially true with Anti-A. This antibody was first observed between the third and fourth week of age. The titer, however, was usually considerably lower than that found for normal antibodies in cattle or humans. Wide variations occur between individuals and for the same individual tested at different times.

7. There is some indication that season of the year may have an influence on titer, but the results are inconclusive.

8. Lactating sows tend to have a lower titer than the same sows in the non-lactating state. This may be the result of rather large quantities of antibody passing into the milk.

9. Anti-A is similar in its reactivity to cattle Anti-J.

10. The presence of normal antigens and antibodies studied is controlled by genetic factors.

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AUTOBIOGRAPHY

I, Kenneth Philip Miller, was born in Rice County, Minnesota, on September 17, 1915. I received my primary education in the public schools of Rice County, and my secondary education in the Northfield High School, Northfield, Minnesota. My undergraduate training was obtained at the University of Minnesota, from which I received the degree of Bachelor of Science in 1939. From the University of Minnesota, I received the Master of Science degree in 1940. While in residence at the University of Minnesota, I acted in the capacity of assistant to Dr. W. E. Peterson in the Dairy Department. In October, 1940, I received an appointment as Instructor at the North Central School and Experiment Station of the University of Minnesota. In July, 1946, I was appointed an assistant professor, and in September, 1952, I transferred to the Southern School and Experiment Station of the University of Minnesota. From July, 1942, to May, 1946, and from January, 1951, to September, 1952, I was on active duty with the United States Army. The University of Minnesota granted me sabbatical leave to study at Ohio State University from July, 1954, through June, 1955.