THE EFFECT OF FEEDING VARIOUS MEMBERS OF THE COLON TYPHOID-ENTERITIDIS GROUP TO ANEMIC AND NON-ANEMIC PIGS

by

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I. INTRODUCTION

It is a well established fact that animals suffering from pathological conditions have less resistance against certain infections than normal animals. Although the condition that we refer to as normal is not necessarily associated with the formation of antibodies, the normal healthy animal is able to resist infection with certain groups of bacteria to a greater degree than the animal that cannot be so classed. With these points in mind, a study was planned involving anemic and non-anemic pigs as subjects.

In a recent publication Craig (1) reported upon data collected by Doyle, Mathews and Whiting which showed that anemic pigs are highly susceptible to subcutaneous injections of material containing Actinomyces necrophorus, while non-anemic pigs receiving the same material showed no lesions. Biester, Murray, McNutt and Purwin (2) show that Ascaris infested pigs are more susceptible to the effects of Salmonella suipestifer than are normal individuals.

Werkman (3), proceeding on the theory that lowered resistance and greater susceptibility may also be due to avitaminosis, definitely showed that rats, rabbits and

pigeons suffered a marked break in their resistance to infection when deprived of certain vitamins. Rats and rabbits suffering from lack of vitamin A were less resistant to infection with anthrax bacilli and certain types of pneumococcus than were normal animals. Pigeons deprived of vitamin B and fed on polished rice succumbed readily to infection while control pigeons resisted infection from the same organisms. However, this same author (4), in another series of experiments and working with the same species of animals, showed that there was practically no difference in agglutinin formation between the normal individuals and those suffering from lack of vitamins A and B.

With the idea that lowered resistance, as a result of anemia, might increase the susceptibility of pigs, several different organisms of the colon-typhoid-enteritidis group were fed to anemic and non-anemic pigs, and tests made of their sera for the presence of agglutinins for the organisms. Salmonella suipestifer was one of these organisms, much work having been done with it using pigs as subjects. Salmonella enteritidis, Salmonella aertrycke (Mutton) and Escherichia coli were the other members of the group used in this work.

II. REVIEW OF LITERATURE

Anemia in young pigs has been described by several investigators. McGowan and Crichton (5), (6), (7) described it in association with iron deficient diets, iron deficiency in the pigs themselves, and in cases of cotton seed poisoning. More recently Doyle, Mathews and Whiting (8), (9) described anemia as a disease occurring in young pigs that were kept inside a central hog house during the first few weeks of life. These investigators have also made an extensive study of the pathology of this disease. The main lesions as described by them occur primarily in the blood, liver, spleen and heart. The liver is very friable due to degenerative processes; the spleen is often firm; while the heart is usually pale, flabby and dilated.

In the investigations that have been reported on anemia in pigs, comparatively little seems to have been done in certain phases of bacteriology and immunology, such as the feeding of organisms and subsequent serological tests. The agglutination test has in most cases been the chief point of attack in studies of susceptibility of pigs to infection with Salmonella suipestifer, but anemia was not studied as a factor.

Giltner (10), (11) has taken advantage of this test for the detection of agglutinins for Salmonella suipestifer in hogs used during the process of serum production, while McClintock, Boxmeyer and Siffer (12) attempted to use it as a test for the diagnosis of hog cholera. Wehrbein (13) also used the agglutination test during the process of serum production. He tested the sera of 100 hyperimmune hogs and concluded that the agglutinins were produced, not as a result of cholera, but as a result of the presence of the organisms in the large amounts of the virus injected. Dinwiddie (14) drew conclusions similar to those of Wehrbein (13) concerning the source of agglutinins in hog cholera antiserum.

Doyle (15) used pigs in his investigations and found they were good agglutinin producers when fed formaldehyde killed cultures of Salmonella suipestifer. The animals were fed large quantities (200 c.c.) of cultures and gave a complete agglutination in a 1-200 dilution and partial agglutination in a 1-500 dilution when tested a week after being fed.

The production of agglutinins by feeding cultures of micro-organisms has been shown by many investigators. The work was confined mostly to the usual laboratory animals such as rats, rabbits and guinea pigs. Tenbroeck

(6) mentions that while much experimental work has been done on agglutinins very little is known about their nature. His investigations showed that a high titer is not an indication of a high degree of immunity but merely that agglutinins are produced during the development of immunity. Doyle (15) indicates in his summary with reference to rabbits, that the agglutination test is more reliable for detecting small amounts of antibodies in the blood of these animals as a result of feeding certain antigens, than is the precipitation or the complement fixation test.

It would seem that further work on pigs suffering from anemia, using the agglutination test, might furnish data of interest.

III. EXPERIMENTAL

In brief, the experimental work consisted of feeding virulent cultures of Salmonella suipestifer, Salmonella aertrycke (Mutton), Salmonella enteritidis and Escherichia coli to young pigs and noting the results. Repeated agglutination tests were made with the serum of each pig at approximately two to three day intervals until the time of death. In these tests four antigens were used. Each was made from the stock cultures used for

feeding. All pigs which died or were killed were cultured. Cultural characteristics and serological reactions were studied on all the organisms isolated. These in turn were compared with the stock cultures which had been fed.

An outlined description of the experimental work follows. Tables II - VIII, summarizing the data obtained from experimental work, are given at the end of this description, also graphs illustrating some of the points recorded in these tables are included in Figures 1 to 5.

A. History of Cultures

No detailed history was secured on most of the organisms used. The Salmonellae were from the collection of Professor E. O. Jordan. The numbers used to identify them correspond to the numbers which accompanied the cultures when received. They are as follows: S. aertrycke 179, S. enteritidis 332 and 550, S. suipestifer 278 and 290. A culture of E. coli was supplied by Dr. W. A. Hagan. This organism was isolated from a typical case of white scours in a calf. It will hereafter be designated as culture H for simplicity of identification. One

culture, each, of S. pullorum and Eberthella sanguinarium were used as antigens. These two organisms were stock cultures in the Veterinary Department of the Purdue University Agricultural Experiment Station for a considerable time. Further history of them is lacking.

B. Groups of Pigs Used.

1. Pigs Used in Feeding Experiments. Seventy small pigs approximately four weeks of age were used in the feeding experiments. Previous to this work, each pig had had hemoglobin readings taken at weekly intervals to determine the amount of hemoglobin in the blood. The last weekly readings taken were used in the present problem to help determine which pigs were anemic and which were not.

The pigs used were farrowed by 12 gilts that had been kept inside a farrowing house during the entire gestation period. The anemic pigs were never given an opportunity to be out of doors, and were confined at all times in pens that had concrete floors and walls. These conditions were provided as they agree as closely as possible with field conditions which tend to induce

anemia, that is, central hog houses, many of which have concrete floors and usually insufficient window space. It might be well to state that these pigs were irradiated during the time previous to this experiment to insure a supply of vitamin D.

For the non-anemic controls, pigs of the same age were used. These, however, had been outside continuously from one day of age and had plenty of sunlight and free access to blue grass pasture, dirt and anything which they might eat while under these conditions. These pigs were brought inside and kept in during the course of the experiment.

Thirteen anemic pigs were fed S. aertrycke 179. Their hemoglobin in grams per 100 c.c. of blood was 7.06 as compared to 15.8 for the four non-anemic controls.

Fifteen anemic pigs with 6.1 grams of hemoglobin and five non-anemic pigs with 15.5 grams were fed cultures of S. enteritidis 332 and 550.

Nineteen anemic pigs were in the group employed for the study of S. suipestifer (cultures 278 and 290). The average hemoglobin reading for these pigs was 6.5 grams. Of this number, there were two pigs, 25 and 26, which did not receive cultures. Their status will be explain-

ed later. The number of non-anemic controls for S. suipestifer feeding was seven, with an average hemoglobin of 17.0 grams.

The average hemoglobin for the seven remaining anemic pigs was 7.04 grams. This group was fed E. coli, H. Non-anemic pigs were not fed this organism as none were available at that time.

Table I. Grouping of pigs as to cultures fed and hemoglobin readings.

Anemic pigs.			Non-anemic pigs.		
Culture fed	Number of pigs fed	Hemoglobin reading	Culture fed	Number of pigs fed	Hemoglobin reading
S.aert.	13	7.06	S.aert	4	15.8
S.ent.	15	6.10	S.ent.	5	15.5
S.spfr.	19	6.50	S.spfr.	7	17.0
E.coli	7	7.04	E.coli		

2. Pigs Used for Special Agglutination Tests. Serum from one pig (59), already accounted for in the group of 70 pigs which were fed the various organisms, was used for a further series of agglutination tests.

An additional group of 22 pigs was selected primarily for the purpose of determining whether their sera contained agglutinins for S. pullorum and E. sanguinarium.

It was thought that this added data on possible agglutinin production for these organisms, in addition to the data obtained from the feeding experiments, might be of interest.

These pigs had been used in a former anemia experiment and were determined definitely anemic at four weeks of age. At the time the cultures were fed to them they were 12 weeks of age. During the intervening period of eight weeks, they had been running in outside yards.

C. Collection of Blood Samples.

During the entire course of the experiment eac litter of pigs was kept in a separate pen. The pigs were driven through a short alley into a room which was used as a bleeding room, and also as a room for feeding the cultures. Both alley and bleeding room had concrete floors. After each group of pigs had traveled this route, the floors were thoroughly hosed and cleaned before other pigs were moved from their pens into the same room.

While each pig was being bled it was held by an assistant. First a small portion of the end of the tail was cut off, then approximately one cubic centimeter of blood was collected in a small vial. After this, the

tail was seared with a hot iron to prevent further hemorrhage.

Blood samples were taken from each pig before the feeding of cultures was begun. In most cases samples were again taken on the second or third day after feeding of the cultures, and the tests were repeated at about two or three day intervals. Tables II-V indicate that this schedule was varied in some instances. Such readjustment was necessary in order to make a more uniform schedule of bleedings so that an equal number would fall on successive days. This testing continued until the time of death of each animal.

D. Preparation and Feeding of Cultures.

Transfers of cultures to be fed were inoculated into flasks containing 100 c.c. of beef infusion broth with a H-ion concentration adjusted to a pH of 7.0 to 7.2, and containing 1.0 percent peptone and 0.5 percent salt. All flasks were incubated at 37.5°C. for 18 to 24 hours. This incubation period produced a very luxuriant growth of all organisms.

Each pig was given 20 c.c. of the broth cultures. All animals were handled in the same manner. Twenty

cubic centimeter, all glass syringes were used for administering the cultures. Pieces of stiff rubber tubing approximately six inches long and one-quarter of an inch in diameter were attached to the ends of the syringes. Small round pieces of wood, six inches long and three quarters of an inch in diameter, were used as mouth specula. A small hole, just large enough to accomodate the tubing, was burned through each midway between the ends. The pigs were held by an assistant and with the aid of the mouth speculum the culture was introduced through the rubber tubing directly into the stomach, or well down the esophagus.

All syringes, tubing and specula were soaked in 10 percent formaldehyde solution between feedings of the different litters. Feedings in most cases were from one to several days apart. This insured all equipment being free from contaminating bacteria. When more than one group was fed on the same day and different organisms were used, a different set of equipment was used for each specie of organism.

The attempt was made to prevent all pigs from nursing for a short time before feeding cultures, as there was much less chance of vomiting after the cultures were fed if given on an empty stomach. Vomiting occurred in

only a very few instances. When it did, the vomitus was cleaned up immediately and the floor disinfected. In these cases a duplicate dose was given. All pigs were kept under close observation for several minutes after feeding. When emesis occurred, it took place in five minutes in practically all cases. During the actual feeding period, all pigs were kept in the bleeding room and remained there until all danger of vomiting was over. Thorough cleaning and disinfection were practiced after each group had been fed cultures. This protected all subsequent groups of pigs entering this room against picking up organisms which had been fed previously.

E. Agglutination Tests.

As soon as the serum separated from the blood samples, taken as described, it was tested against the antigens made from the four stock species of organisms. The S. aertrycke antigen was made from strain 179; S. enteritidis antigen from strain 550; S. suipestifer from strain 290, and E. coli antigen from culture H. It may be of interest to mention the rapidity with which the serum separates in anemic blood. Practically all pigs with low hemoglobin have much more serum in proportion to red cells than nor-

mal pigs. As a result of this, a rapid separation takes place. In most instances the agglutination test could be set up within half an hour after the sample was collected.

In testing the blood before the cultures were fed, and in all subsequent tests after feeding, the agglutination test was used exclusively. Antigens of the organisms used for feeding the pigs were prepared by inoculating them on two percent beef infusion agar containing one percent peptone and 0.5 percent salt. The H-ion concentration was adjusted within a range of pH 7.0 to 7.2. Ordinary pint flasks were used for growing the cultures. These were incubated for 48 hours, after which time the maximum growth was usually reached. The surface of the growth was then covered with formalized saline. This was prepared by adding 0.85 percent of chemically pure sodium chloride and 0.25 percent formalin to the required amount of distilled water. Liter amounts were usually made up at one time. This would require 8.5 grams of salt and 2.5 c.c. of formalin to the 1000 c.c. of distilled water.

This formalized saline solution was allowed to remain in contact with the organisms for approximately eight to ten hours. This had a two-fold action. It would loosen the growth and at the same time kill the or-

ganisms. Sterility tests were run on each antigen by inoculating a loopful on an agar slant and incubating it over night. In all cases the slants remained sterile. After this procedure, the suspension of organisms was poured into a flask and the turbidity adjusted to conform with tube Number 2 of the McFarland nephelometer. The antigen was then strained through several layers of absorbent cotton after which it was bottled. Antigens prepared in this manner were very satisfactory. When a new amount was made, it was compared with a tested lot which had previously been used and proved to be satisfactory.

In setting up tests, the serum was pipetted directly into agglutination tubes in amounts of 0.1 c.c., 0.05 c.c., 0.02 c.c. and 0.01 c.c. The antigen was next added to each tube in quantities of 1 c.c. A set of four dilutions was made for each antigen. These serum antigen combinations made dilutions of approximately 1-10, 1-20, 1-50 and 1-100 respectively. All tubes were shaken thoroughly after the antigen was added and then incubated at 37.5°C. over night. The total incubation period usually averaged 16 to 18 hours.

After agglutinins appeared and when necessary to make higher dilutions a 1-10 dilution of the original

serum was made with formalized saline. With this dilution the original procedure was repeated using 0.05 c.c., 0.02 c.c. and 0.01 c.c. which gave dilutions of 1-200, 1-500 and 1-1000 respectively after the addition of the antigen. In a few instances it was necessary to use a 1-100 standard dilution of the original serum in order to get dilutions of 1-2000, 1-5000 and 1-10,000.

F. Bacteriological Examination.

After death, each pig was examined and cultures made from the heart, liver and spleen, using agar slants. The agar used was identical with that used in making the antigens. Each tube was incubated for approximately 18 hours after which it was examined for bacterial growth. All cases which yielded growth were subcultured and then subjected to the various bacteriological and morphological tests. At this same time each pig was examined for lesions of anemia and any other lesions that might have resulted from the feeding of the various organisms.

G. Agglutinin Absorption Tests.

Agglutinin absorption tests were made with all the organisms that were isolated from the pigs. In doing

this phase of the work sera from two representative pigs were selected. Pig 46 had been fed S. aertrycke 179, while pig 20 received S. suipestifer 278. The former had an agglutinating titer of 1-500 and the latter 1-1000. Pig 46 was killed on the 26th day after feeding, and pig 20, at the point of death on the 13th day, was killed. Blood was collected from each and the sera finally preserved by adding 0.25 percent formalin.

The absorbed serum was prepared for this test very much in the same manner as Andrews (17) prepared his mono-specific serum. Five cubic centimeters of each serum were placed in separate sterile bottles. To the S. suipestifer serum there were added 45 c.c. of an extremely heavy suspension of S. aertrycke 179, previously prepared by washing off the growth from six pint flasks containing 48 hour agar cultures. To five c.c. of the S. aertrycke serum a similar suspension of S. suipestifer 290 was added. Each was incubated for 48 hours during which time they were frequently shaken.

After the incubation period they were centrifuged at a high rate of speed until the supernatant fluid was clear. This usually required about two hours. The resulting supernatant fluid, a 1-10 dilution, was pipetted off and

used against antigens prepared from the various organisms isolated from the pigs and also against the stock antigens. Antigens prepared from the isolated organisms were made in exactly the same manner as were the stock antigens.

Dilutions of 1-50, 1-100, 1-200 and 1-500 were made in all of these tests by adding 0.2 c.c., 0.1 c.c., 0.05 c.c. and 0.01 c.c. of the absorbed serum respectively to each of four tubes and then adding the antigens to bring the volume of each to one c.c. S. aertrycke and S. suipestifer absorbed sera were both used in this manner.

H. Special Agglutination Tests.

As a further check on the identification of the isolated organisms a series of agglutination tests were made using serum from pig 59. This pig was fed S. enteritidis 550. It was killed on the fourteenth day after feeding, at which time the serum was collected. Bacteriological examination of the organs of this pig showed them to be sterile. All stock antigens and isolated organism antigens were used with this serum in dilutions of 1-10, 1-20, 1-50 and 1-100.

The 22 pigs to which no cultures were fed, were tested for S. pullorum and E. sanguinarium agglutinins. The sera from these pigs were also tested against antigens of

S. aertrycke, S. enteritidis, S. suipestifer and E. coli, the same strains used in all other agglutination tests. Likewise, dilutions of 1-10, 1-20, 1-50 and 1-100 were used.

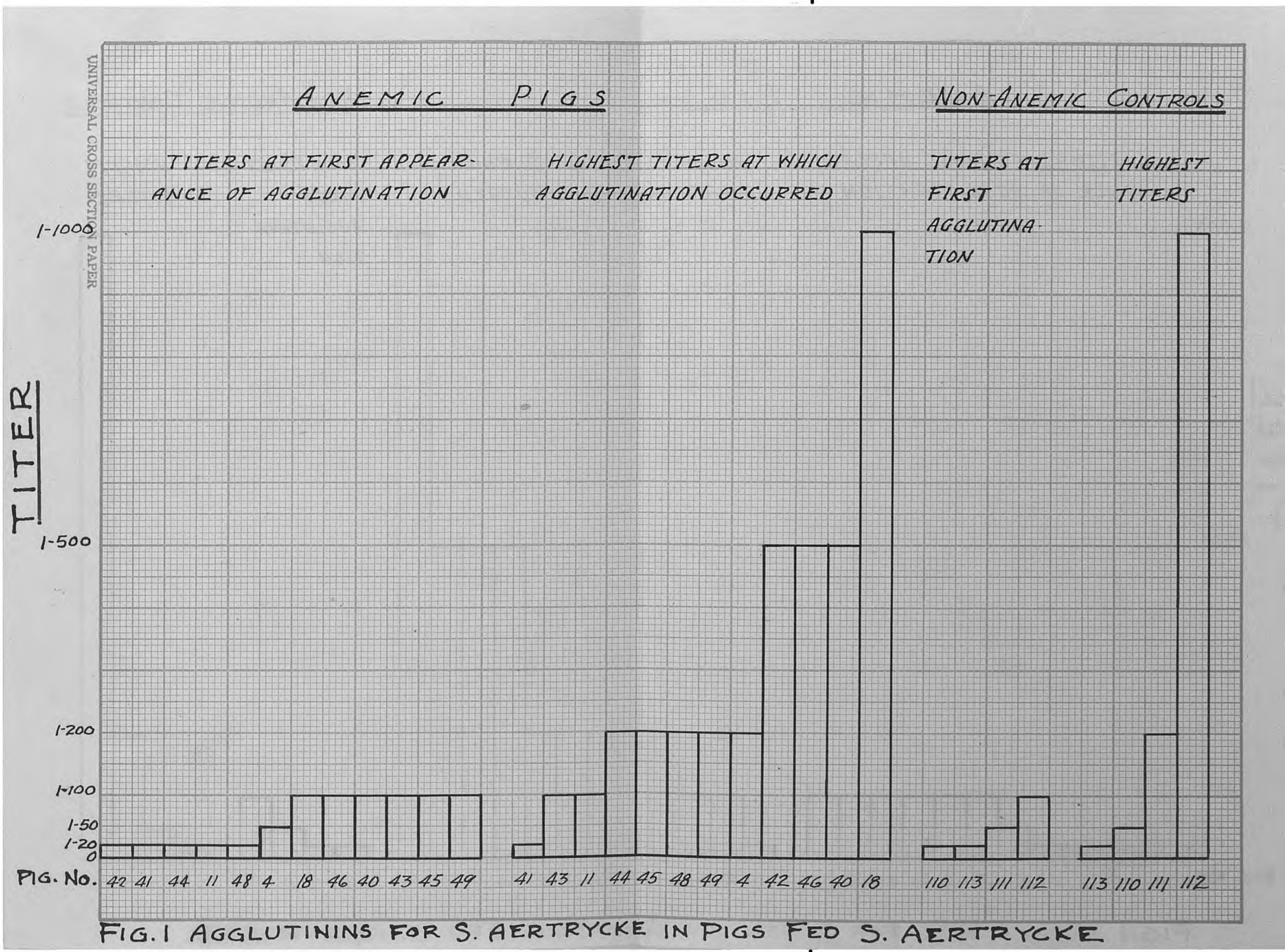

FIG. 1 AGGLUTININS FOR S. AERTRYCKE IN PIGS FED S. AERTRYCKE

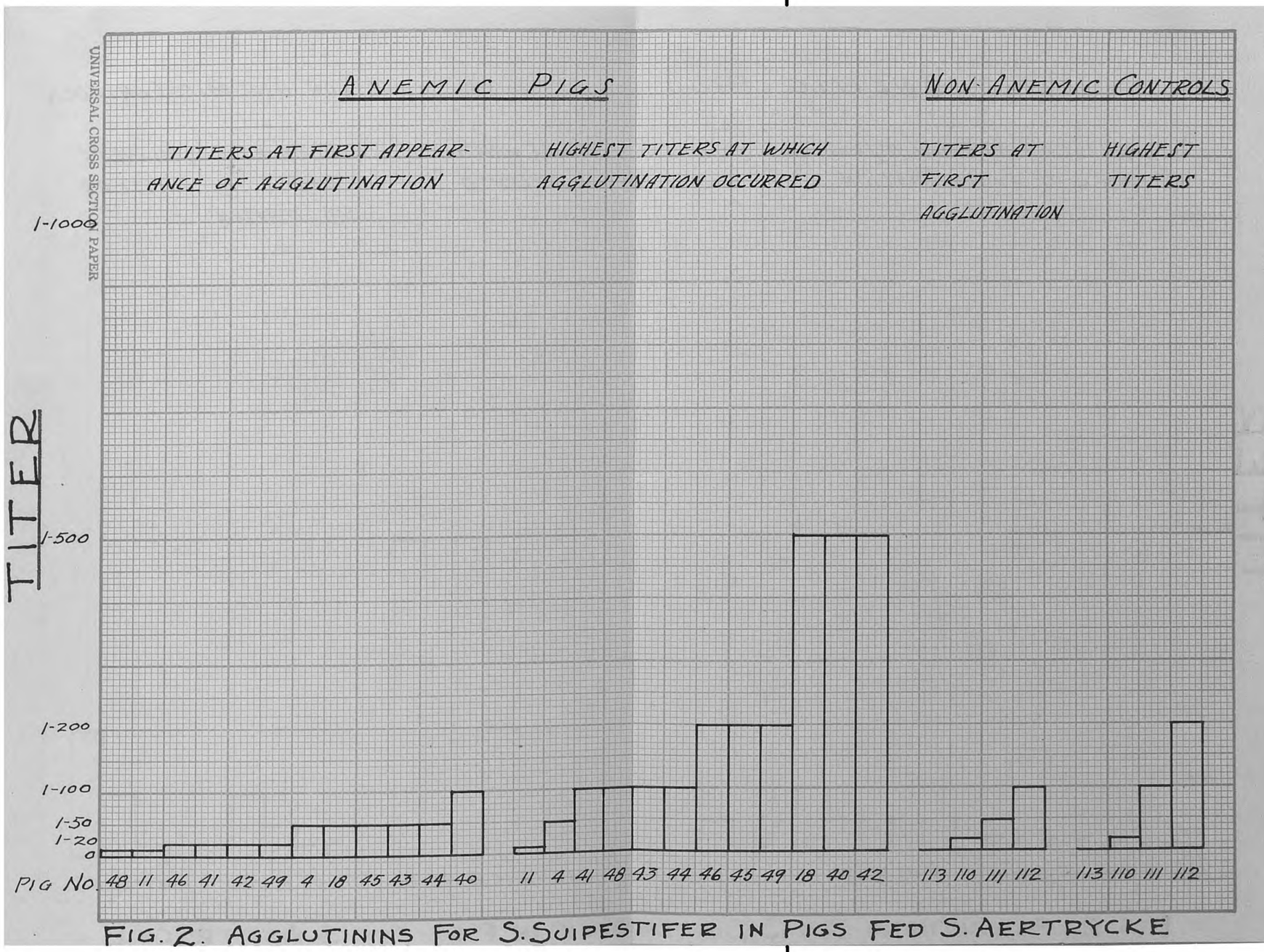

FIG. 2. AGGLUTININS FOR S. SUIPESTIFER IN PIGS FED S. AERTRYCKE

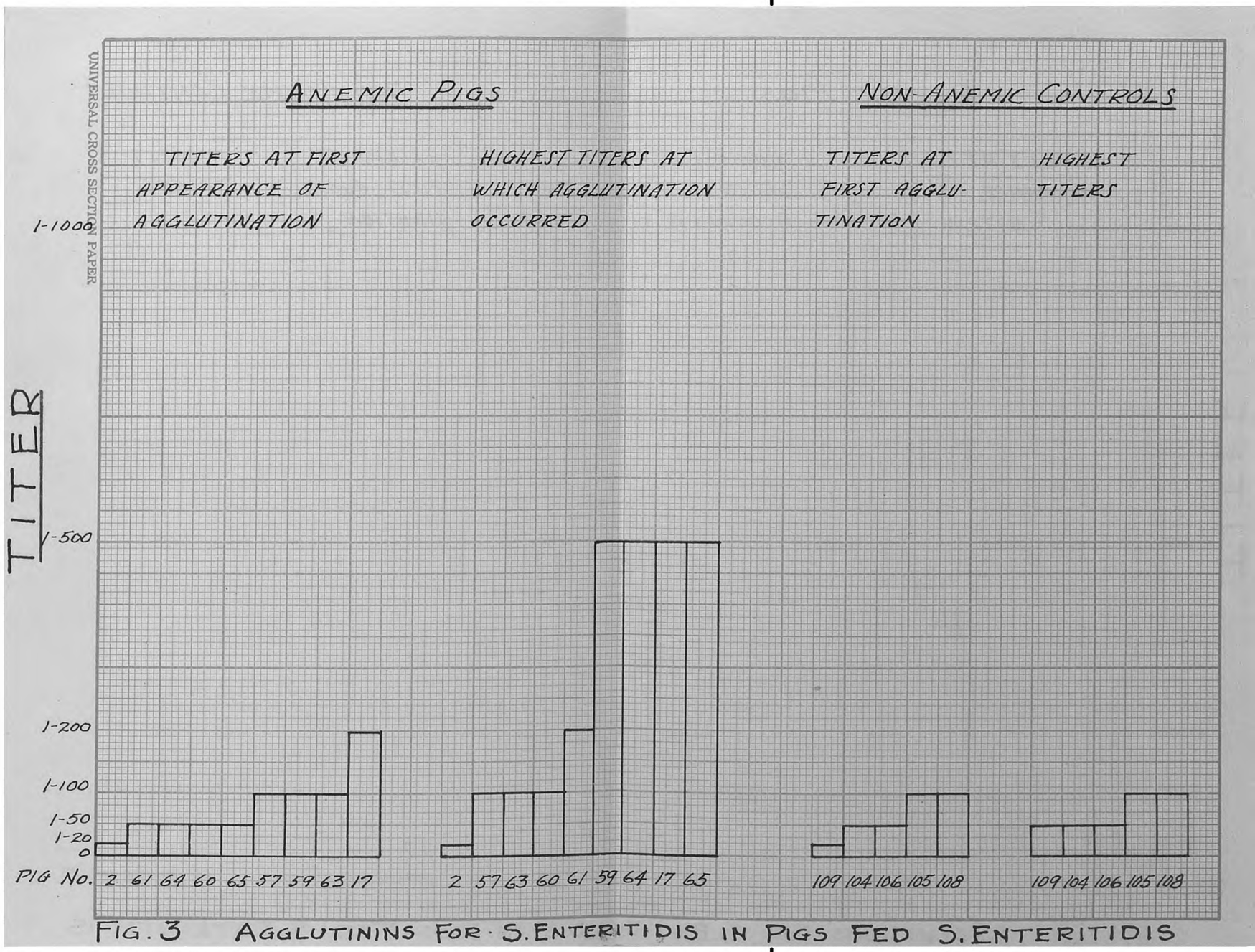

FIG. 3 AGGLUTININS FOR S. ENTERITIDIS IN PIGS FED S. ENTERITIDIS

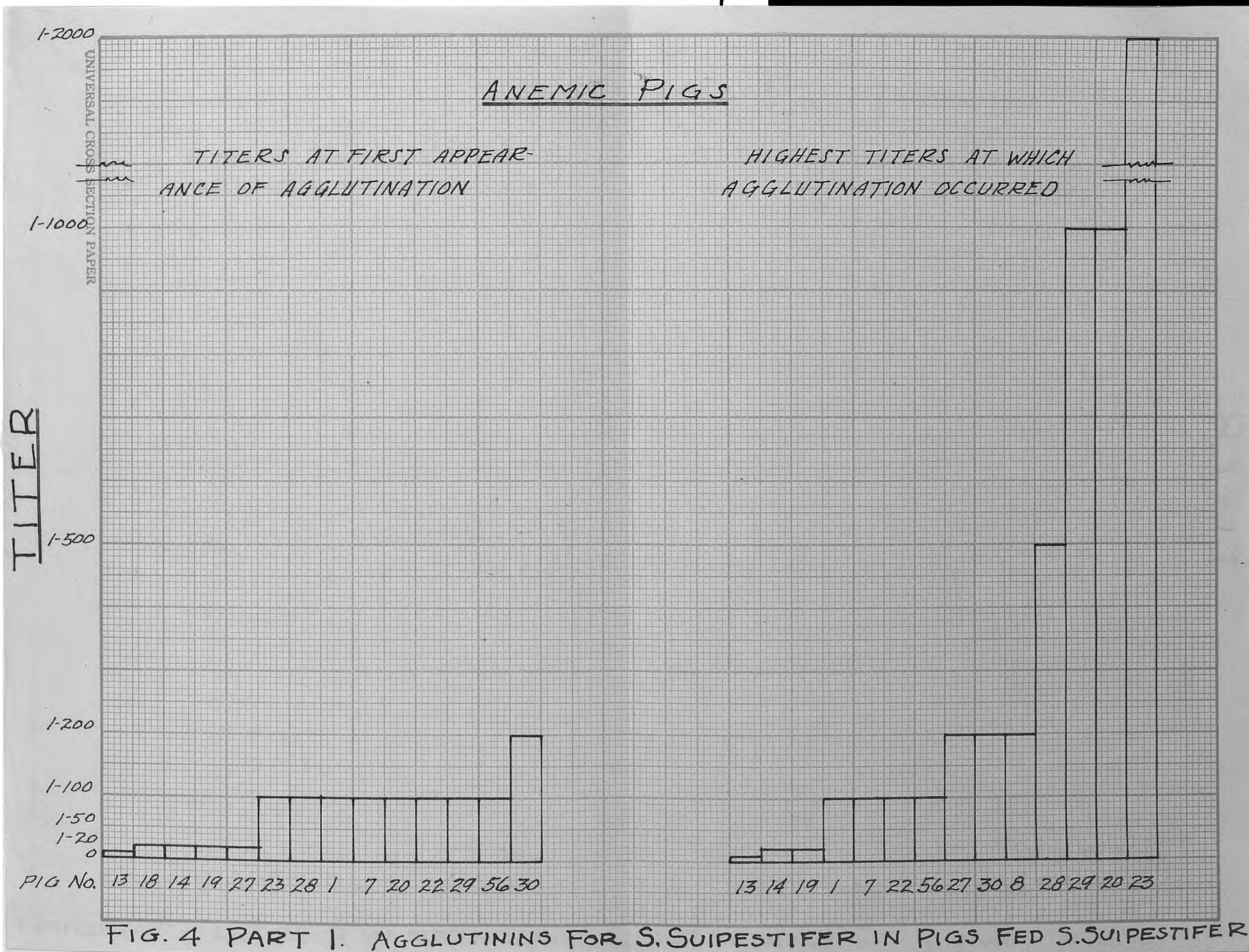

FIG. 4 PART 1. AGGLUTININS FOR S. SUIPESTIFER IN PIGS FED S. SUIPESTIFER

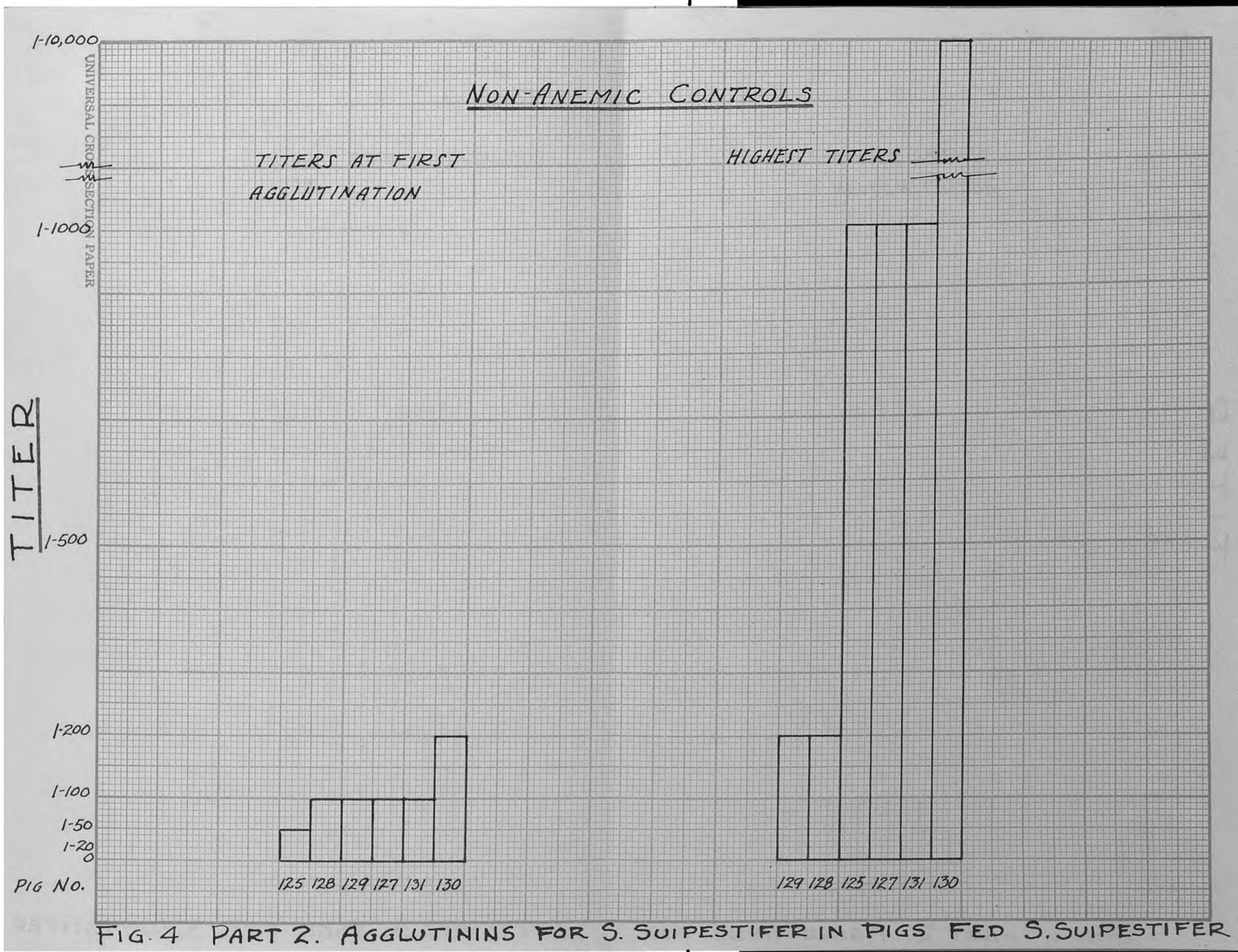

FIG. 4 PART 2. AGGLUTININS FOR S. SUIPESTIFER IN PIGS FED S. SUIPESTIFER

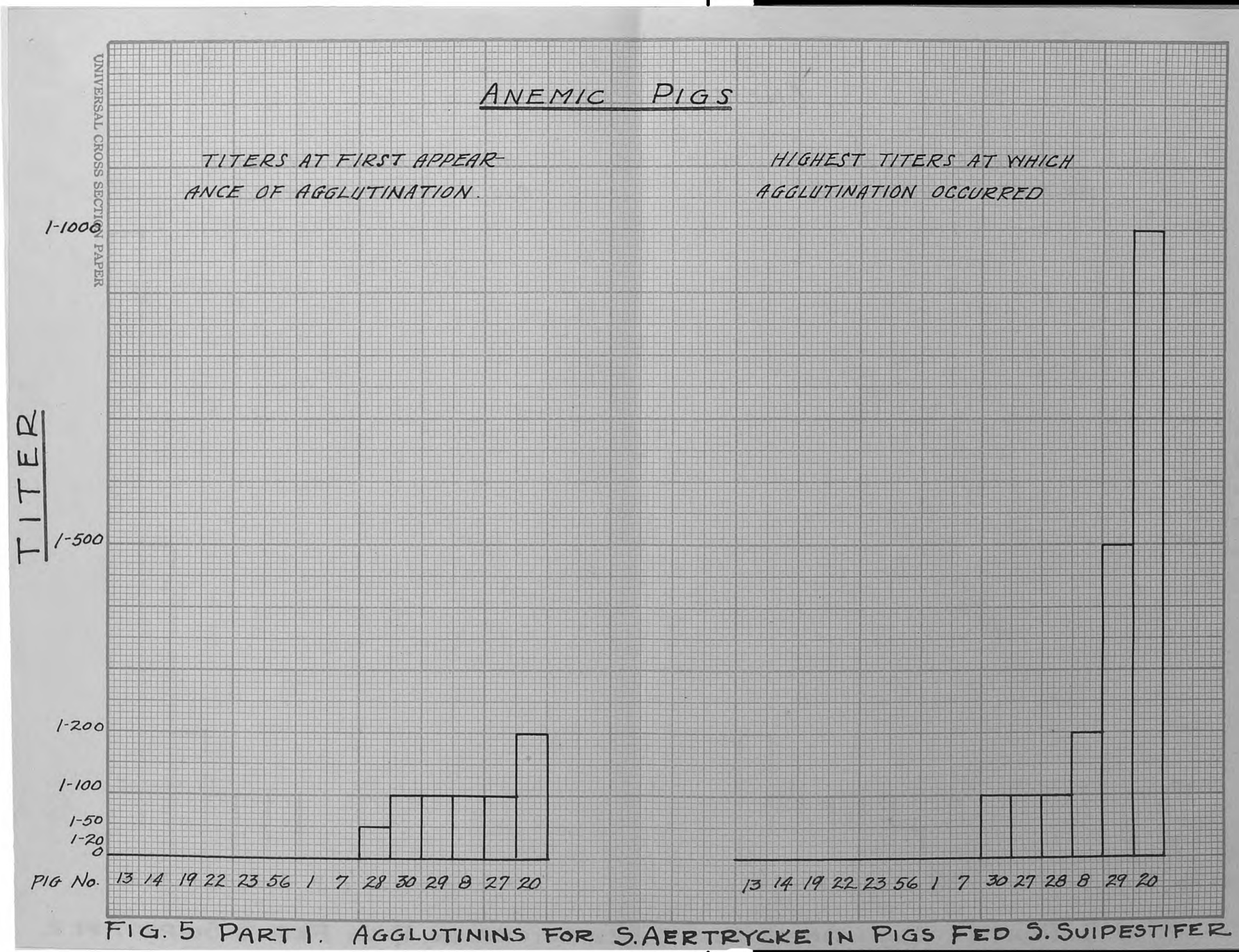

FIG. 5 PART I. AGGLUTININS FOR S. AERTRYCKE IN PIGS FED S. SUIPESTIFER

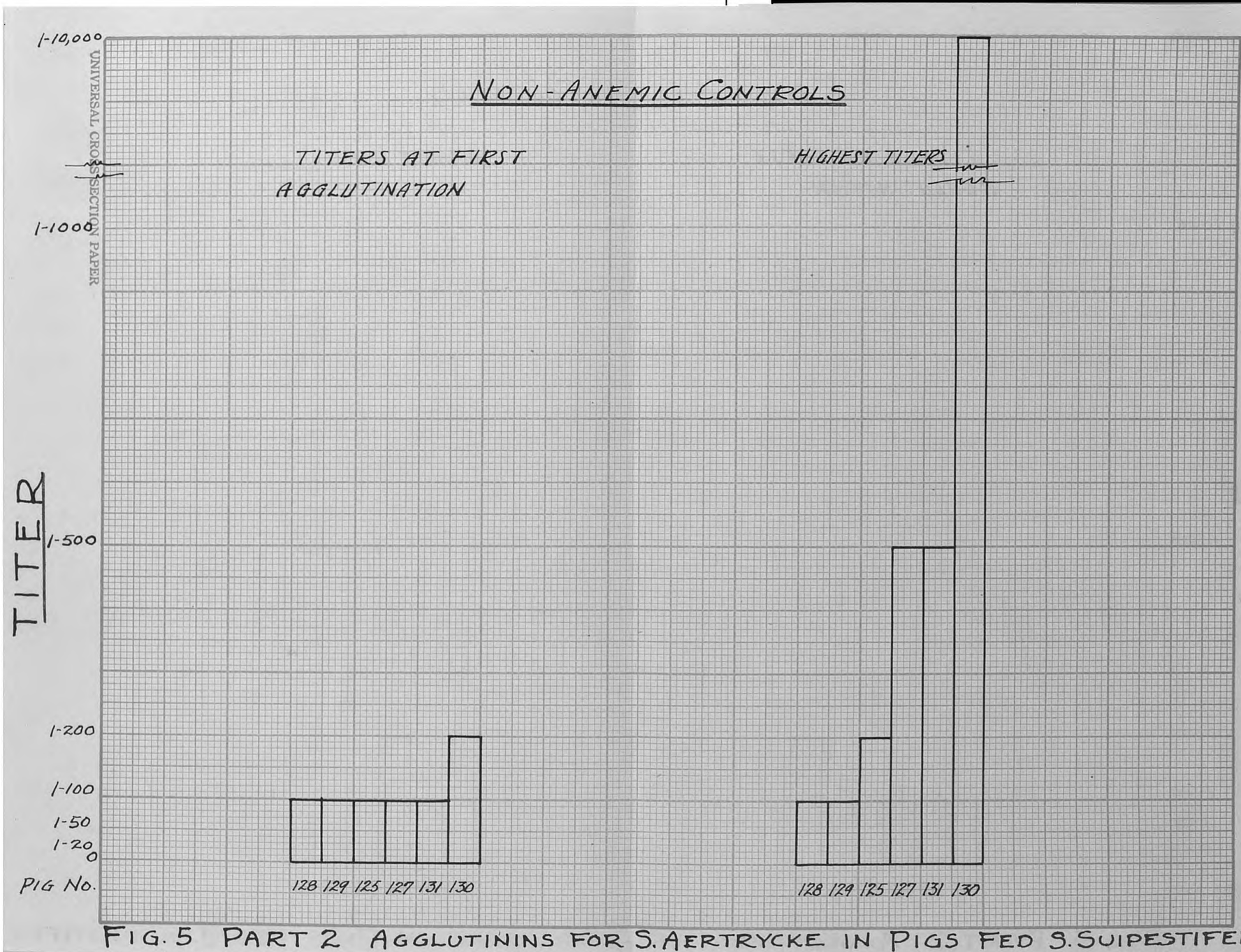

FIG. 5 PART 2 AGGLUTININS FOR S. AERTRYCKE IN PIGS FED S. SUIPESTIFER

Table II. Results Obtained on Feeding Pigs S. aertrycke, 179.

Pig no.	Agglutinins before feeding	Appearance of agglutinins in days after feeding																Remarks
		2	4	5	6	8	10	11	12	14	16	17	18	20	23	26	33	36	
4	-	-	-		A50 S50	A100 S50	A100 S20		A100 S10	A200 S20	A100		A200	A100					Destroyed 20th day Anemia
9																			Died 18 hours Anemia
11	-	-	-		-	A20	A100		A50 S10	A50 S10									Destroyed 14th day Anemia
18	-	-	-		A100 S50	A500 S100	A1000 S500												Destroyed 10th day Anemia
40	-	-	-		-	A100 S100	A500 S500		A200 S500	A50 S50									Destroyed 14th day Anemia
41	-	-	-		A20 S20	A20 S20	S50		S100	A10 S50									Destroyed 14th day Anemia
42	-	-	A20		-	A20 S20		A100 S100		A100 S100		A100 S100		A500 S200	A500 S500	A500 S200			Destroyed 26th day Anemia
43	-	-	-		-	A100		A50		A100		A100 S50		A100 S100	A100 S100				Destroyed 23rd day Anemia
44	-	-	-		A20	A20		A100		A100 S50		A200 S100		A200 S50	A200 S100	A200 S100			Destroyed 26th day Anemia
45	-	-	-		-	A100 S50		A100 S50		A200 S100		A200 S100		A200 S100	A200 S200	A200 S100			Destroyed 26th day Anemia
46	S20	S20	S50		A100 S100	A200 S200		A500 S200		A200 S200		A200 S200		A500 S200	A200 S100	A500 S200			Destroyed 26th day Anemia
48	-	-	-		-	A20 S10		A100 S50		A200 S100		A100 S50		A50 S20	A50 S20				Destroyed 23rd day Anemia
49	-	-	-		-	A100 S20		A100 S20		A200 S50		A200 S100		A200 S200	A100 S100		A20		Destroyed 23rd day Anemia
110*	-	-	-	-		-		-		A20		A50		A50			A20 S20		Destroyed 33rd day Normal
111*	-	-	-	-		-		A50		A100 S50		A200 S100		A200 S100			A100 S100	A100 S100	Destroyed 36th day Normal
112*	-	-	-	A100		A100 S100		A1000 S200		A500 S200		A500 S200		A1000 S200			A200 S200		Destroyed 33rd day Normal
113*	-	-	-	-	-	-		-		-		A20		A20			-		Destroyed 33rd day Normal

A and S - Presence of agglutinins for S. aertrycke and S. suipestifer respectively.
Numbers appearing after letters represent titer of serum.
*-Non-anemic pigs.

Table III. Results Obtained on Feeding Pigs S. enteritidis, 332 and 550.

Pig no.	Agglutinins before feeding.	Appearance of agglutinins in days after feeding																Remarks.
		2	4	6	8	10	11	12	14	16	17	19	20	22	25	28	31	33	
2a	-	-	-	E20	E20		-												Destroyed 11th day. Anemia.
6a	-	-																	Died 2nd day. Anemia.
16a	-	-																	Died 2nd day. Anemia.
17a	-	-	-	E200	E200	E500													Destroyed 10th day. Anemia.
37	-	-																	Died 2nd day. Anemia.
39	-																		Died 18 hours. Anemia.
57	-	-	E100																Died 4th day. Anemia.
59	-	-	E100	E100	E100	E500		E500	E500										Destroyed 14th day. Anemia.
60	-	-	-	E50	E50	E100		E50	E20	E50		E50		E50	E50	E50	E50		Destroyed 32nd day. Anemia.
61	-	-	E50	E50	E50	E100		E100	E100	E200		E200		E200	E200	E100	E100		Destroyed 32nd day. Anemia.
63	-	-	E100	E100	E100														Died 8th day. Anemia.
64	-	-	E50	E100	E100	E500		E200	E200	E500		E200		E200	E200	E50			Destroyed 29th day. Anemia.
65	-	-	-	E50	E100	E200		E200	E100	E500		E200		E100	E100	E20			Destroyed 29th day. Anemia.
66	-	-																	Died 2nd day. Anemia.
69	-	-	-	-	-	-		-	-			-							Destroyed 19th day. Anemia.
104*	-	-	-	-	E50		E50		E50		E50		E50					E50	Destroyed 36th day. Normal.
105*	-	-	-	-	E100		E50		E50		E50		E100					E50	"
106*	-	-	-	-	E50		E20		E50		E50		E50					E20	"
108*	-	-	-	-	E100		E100		E50		E50		E50					E50	"
109*	-	-	-	-	E20		E20		E50		E20		E20					E20	"

E - Presence of agglutinins for S. enteritidis.
Numbers appearing after letters represent titer of serum.
a - Pigs fed culture 332.
* - Non-anemic pigs.

Table IV. Results Obtained on Feeding Pigs S. suipestifer, 278 and 290.

Pig no.	Agglutinins before feeding.	Appearance of agglutinins in days after feeding: 3	5	4	5	6	8	10	11	12	14	16	17	18	20	22	25	31	Remarks.
13a	-	-	-			S10													Died 6th day Anemia, colitis
14a	-		-			S20													Died 6th day Anemia, colitis
15a	-		-		-														Died 5th day Anemia, colitis
19a	-	-		-		S20													Died 6th day Anemia, colitis
20a	-	-		-		S100	S1000	S1000 A200		S1000 A1000									Died 13th day Anemia, colitis
22a	-	-		-		S100													Died 6th day Anemia, colitis
23a	-	-		-		S100	S1000	S2000											Died 10th day Anemia, colitis
54a	-	-		-															Died 4th day Anemia, colitis
56a	-	-		-			S100												Died 8th day Anemia, colitis
67a	-																		Accidental injury, destroyed 24 hours, anemia.
1	-	-		S100															Died 4th day Anemia
7	-	-		S100															Died 4th day Anemia
8	-	-		S20			S10	S100 A100		S200 A200									Died 12th day Anemia, colitis
25	-	-		-		-	-	-		-	-	S50		S100	S50	S50	-	-	Destroyed 34th day Anemia
26	-	-		-		-	-	S100 A100		S100 A100	S100 A100	S1000 A500		S100 A1000	S500 A500	S500 A500	S1000 A1000	S1000 A1000	Destroyed 34th day Anemia
27	-	-		-		S20	S20	S50		S20	S50	S100		S200	S200	S200 A100	S100 A100	S100	Destroyed 34th day Anemia
28	S100	S100 A50		S500 A100															Died 4th day Anemia, colitis
29	-	-		-		-	S100 A100	S200 A100		S1000 A200	S1000 A500	S1000 A500		S1000 A500	S1000 A500	S500 A200	S200 A200	S200 A100	Destroyed 34th day Anemia
30	-	-	S200 A100																Died 3rd day Anemia
125*	-	-			S50		S200 A100		S200 A100		S1000 A200		S200 A100		S200 A50				Destroyed 20th day Normal
126*	-	-																	Died 2nd day Gastro enteritis
127*	-	-			-		S100 A100		S1000 A500		S1000 A500		S500 A200		S500 A100				Destroyed 40th day Normal
128*	-	-			S100 A100		S200x												Died 8th day Colitis
129*					S100 A100		S200x												Died 8th day Colitis
130*	-	-			S200 A200		S1000 A1000		S10,000 A10,000										Destroyed 11th day Colitis
131*	-	-			-		S100 A100		S1000 A500		S1000 1200		S500 A200		S200 A100				Destroyed 40th day Colitis

S and A-presence of agglutinins for S. suipestifer and S. aertrycke respectively.
Numbers appearing after letters represent titer of serum.
a - Pigs fed culture 278, remaining pigs fed 290.
x - Sufficient serum for S. suipestifer test only.
* - Non-anemic pigs.

Table V. Results Obtained on Feeding Pigs E. coli, H.

Pig no.	Agglutinins before feeding	Appearance of agglutinins in days after feeding						Remarks
		3	6	9	12	15	18	
31	-	H100	H100	H50	H50	H50	H20	Destroyed 18th day, anemia.
32	-	H20	H50	-	H50	H50	H50	"
33	-	H20	H100	H20	H100	H20	H50	"
34	H20	H50	H100	H100	H100	H100		Destroyed 15th day, anemia.
35	-	H100	H100	-	H100	H20	H20	Destroyed 18th day, anemia.
51	-	-	-					Destroyed 6th day, anemia.
55	-	-	-					"

H - Presence of agglutinins for E. coli.
Numbers appearing after letters represent titer of serum.

Table VI. Cultural Characteristics of Isolated Organisms.

Pig no.	Organism fed	Gram stain	Motility	Gelatin liquefied	Indol Production	Lead acetate agar blackened	Dextrose Maltose	Lactose Saccharose	Arabinose	Xylose	Dulcite	Inosite	Litmus milk
9	179	-	+	-	-	+	AG	-	AG	AG	AG	-	Sl. acid then moderate alk. 48-96 hrs.
11	179	-	+	-	-	-	AG	-	-	AG	-	-	Sl. acid then faint alk. 48-96 hrs.
40	179	-	+	-	-	-	AG	-	-	AG	-	-	Sl. acid then faint alk. 48-96 hrs.
6	332	-	+	-	-	+	AG	-	AG	AG	AG	-	Acid then extreme alk. 48-96 hrs.
16	332	-	+	-	-	+	AG	-	AG	AG	AG	-	Acid then extreme alk. 48-96 hrs.
17	332	-	+	-	-	-	AG	-	-	AG	-	-	Sl. acid then faint alk. 48-96 hrs.
37	550	-	+	-	-	+	AG	-	AG	AG	AG	-	Acid then extreme alk. 48-96 hrs.
39	550	-	+	-	-	+	AG	-	AG	AG	AG	-	"
57	550	-	+	-	-	+	AG	-	AG	AG	AG	-	"
63	550	-	+	-	-	+	AG	-	AG	AG	AG	-	"
66	550	-	+	-	-	+	AG	-	AG	AG	AG	-	"
13	278	-	+	-	-	-	AG	-	-	AG	-	-	Sl. acid then faint alk. 48-96 hrs.
14	278	-	+	-	-	-	AG	-	-	AG	-	-	"
15	278	-	+	-	-	-	AG	-	-	AG	-	-	"
19	278	-	+	-	-	-	AG	-	-	AG	-	-	"
20	278	-	+	-	-	-	AG	-	-	AG	-	-	"
22	278	-	+	-	-	-	AG	-	-	AG	-	-	"
23	278	-	+	-	-	-	AG	-	-	AG	-	-	"
54	278	-	+	-	-	-	AG	-	-	AG	-	-	"
56	278	-	+	-	-	-	AG	-	-	AG	-	-	"
1	290	-	+	-	-	-	AG	-	-	A	-	-	"
7	290	-	+	-	-	-	AG	-	-	AG	-	-	"
8	290	-	+	-	-	-	AG	-	-	AG	-	-	"
28	290	-	+	-	-	-	AG	-	-	AG	-	-	"
30	290	-	+	-	-	-	AG	-	-	AG	-	-	"
126	290	-	+	-	-	-	AG	-	-	AG	-	-	"
128	290	-	+	-	-	-	AG	-	-	AG	-	-	"
129	290	-	+	-	-	-	AG	-	-	AG	-	-	"
130	290	-	+	-	-	-	AG	-	-	AG	-	-	"

Stock Organism Controls

		-	+	-	-	+	AG	-	AG	AG	AG	-	Sl. acid then moderate alk. 48-96 hrs.
		-	+	-	-	+	AG	-	AG	AG	AG	-	Acid then extreme alk. 48-96 hrs.
		-	+	-	-	+	AG	-	AG	AG	AG	-	"
		-	+	-	-	-	AG	-	-	AG	-	-	Sl. acid then faint alk. 48-96 hrs.
		-	+	-	-	-	AG	-	-	AG	-	-	"
		-	+	-	+	-	AG	Lactose AG Sacch. -					Acid coagulation. 24 hrs.

AG - Acid, gas.

Table VII. Agglutinin Absorption Tests.

Antigen	Organism fed	No. 20 (S. spfr. serum) absorbed by No. 179 S. aert. antigen					No. 46 (S. aert. serum) absorbed by No. 290 S. spfr. antigen				
		1-50	1-100	1-200	1-500	Control	1-50	1-100	1-200	1-500	Control
9	179	-	-	-	-	-	+++	+++	++	++	-
11	179	+++	+++	+++	+++	-	-	-	-	-	-
40	179	+++	+++	+++	+++	-	-	-	-	-	-
6	332	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
16	332	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
17	332	++++	++++	++++	++++	-	-	-	-	-	-
37	550	-	-	-	-	-	-	-	-	-	-
39	550	-	-	-	-	-	-	-	-	-	-
57	550	-	-	-	-	-	-	-	-	-	-
63	550	-	-	-	-	-	-	-	-	-	-
66	550	-	-	-	-	-	-	-	-	-	-
13	278	++++	++++	++++	++++	-	-	-	-	-	-
14	278	++++	++++	++++	++++	-	-	-	-	-	-
15	278	++++	++++	++++	++++	-	-	-	-	-	-
19	278	++++	++++	+++	+++	-	-	-	-	-	-
20	278	++++	++++	++++	++++	-	-	-	-	-	-
22	278	++++	++++	+++	+++	-	-	-	-	-	-
23	278	++++	++++	++++	++++	-	-	-	-	-	-
54	278	++++	++++	+++	+++	-	-	-	-	-	-
56	278	++++	++++	+++	+++	-	-	-	-	-	-
1	290	++++	++++	++++	++++	-	-	-	-	-	-
7	290	++++	++++	++++	++++	-	-	-	-	-	-
8	290	++++	++++	++++	++++	-	-	-	-	-	-
28	290	++++	++++	+++	+++	-	-	-	-	-	-
30	290	++++	++++	+++	+++	-	-	-	-	-	-
126	290	++++	++++	++++	++++	-	-	-	-	-	-
128	290	++++	++++	++++	++++	-	-	-	-	-	-
129	290	++++	++++	++++	++++	-	-	-	-	-	-
130	290	++++	++++	++++	++++	-	-	-	-	-	-
Stock Antigens											
179		-	-	-	-	-	+++	+++	+++	+++	-
332		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
550		-	-	-	-	-	-	-	-	-	-
278		++++	++++	++++	++++	-	-	-	-	-	-
290		++++	++++	++++	++++	-	-	-	-	-	-

Table VIII. Special Agglutination Test.

Antigen	Organism fed	Serum of Pig 59 fed S. enteritidis, 550				
		1-10	1-20	1-50	1-100	Control
9	179	-	-	-	-	-
11	179	-	-	-	-	-
40	179	-	-	-	-	-
6	332	+++	+++	+++	+++	+++
16	332	++++	++++	++++	++++	++++
17	332	-	-	-	-	-
37	550	++++	++++	++++	++++	-
39	550	++++	++++	++++	++++	-
57	550	++++	++++	++++	++++	-
63	550	++++	++++	++++	++++	-
66	550	++++	++++	++++	++++	-
13	278	-	-	-	-	-
14	278	-	-	-	-	-
15	278	-	-	-	-	-
19	278	-	-	-	-	-
20	278	-	-	-	-	-
22	278	-	-	-	-	-
23	278	-	-	-	-	-
54	278	-	-	-	-	-
56	278	-	-	-	-	-
1	290	-	-	-	-	-
7	290	-	-	-	-	-
8	290	-	-	-	-	-
28	290	-	-	-	-	-
30	290	-	-	-	-	-
126	290	-	-	-	-	-
128	290	-	-	-	-	-
129	290	-	-	-	-	-
130	290	-	-	-	-	-
Stock Antigens						
179		-	-	-	-	-
332		+++	+++	+++	+++	+++
550		++++	++++	++++	++++	-
278		-	-	-	-	-
290		-	-	-	-	-

IV. DISCUSSION OF RESULTS.

A. Agglutination Tests.

Tables II, III, IV and V give a summary of results of the agglutination tests which were applied to 54 anemic and 16 non-anemic pigs. Some interesting facts were brought out from an examination of these tables.

In all instances in which agglutinins are reported present on the day the pigs died or were killed, the blood was taken from the heart after the cultures were made. In case a pig died during the night, the serum separated in nearly all instances so that it would be massaged from the heart through the opening made for culturing. A short run in the centrifuge in most cases produced a clear non-hemolyzed serum.

1. Results of Tests on 17 Pigs Fed S. aertrycke. Seventeen pigs in all were fed S. aertrycke. Pig 9 of the anemic group which died of hog "flu" 18 hours after feeding the culture will not be included in the discussion. The remaining group consisted of 12 anemic pigs, and four non-anemic controls. The results of this feeding are recorded in Table II, and graphic illustrations

of some of the points in this table are contained in Figures 1 and 2.

From Table II it is seen that after the feeding of S. aertrycke, anemic pigs showed the presence of agglutinins earlier than non-anemic pigs. Agglutinins for S. aertrycke appeared in the 12 anemic pigs in all cases by the eighth day. In seven of the 12 cases, these agglutinins were present by the sixth day. In the group of four non-anemic controls, only one showed agglutinins for S. aertrycke by the eighth day. The order of appearance of the agglutinins for these pigs was as follows: One on the fifth day, one on the eleventh day, one on the fourteenth day and one on the seventeenth day respectively.

Cross agglutination with S. suipestifer occurred as indicated in these results. Here also, anemic pigs showed an earlier appearance of the minor agglutinin (S. suipestifer). In the group of 12 anemic pigs, agglutinins for S. suipestifer appeared by the eighth day after feeding in eight cases. Pig 46 showed agglutinins before feeding was begun, while pigs 11, 43 and 44 showed them on the twelfth, seventeenth, and fourteenth days respectively. Of the four non-anemic controls only one showed these agglutinins by the eighth day, the order of appearance be-

ing as follows: One on the eighth day, one on the fourteenth day, and one on the thirty-second day. One control pig showed no agglutinins for S. suipestifer before it was killed on the thirty-second day.

In the twelve anemic pigs, four showed agglutinins for S. aertrycke first, seven showed the two agglutinins simultaneously, and one showed agglutinins for S. suipestifer first. In the three control pigs showing cross agglutination, S. aertrycke agglutinins appeared first.

Table II was studied for comparisons between titers at first appearance of agglutination and the highest titers in which agglutination occurred. The length of time before appearance of agglutinins is not considered here. It may be seen from Figure 1 that sera of six of the twelve anemic pigs agglutinated S. aertrycke antigen in a 1-100 dilution. For the other six, the range was from 1-20 to 1-50. This comparison is of serum dilutions when agglutinins first appeared. The four controls at first appearance of agglutinins showed titers ranging from 1-20 to 1-100.

In subsequent tests, sera from many of the pigs showed agglutination in still higher dilutions. A comparison of these highest dilutions follows. From Figure 1 it is

seen that of the 12 anemic pigs, sera of eight caused agglutination in a dilution range of 1-200 to 1-500. Serum of pig 18 agglutinated in one test in a 1-1000 dilution. The range of highest serum dilutions for the four non-anemic controls is wide, being from 1-20 to 1-1000.

Figure 2 shows a comparison of the dilutions at which agglutinins for S. suipestifer appeared after S. aertrycke was fed. In the twelve anemic pigs, the first serum dilutions causing agglutination ranged from 1-10 to 1-100, six being from 1-10 to 1-20, and six in a range of 1-50 to 1-100. In the four controls the first serum dilutions in which agglutination occurred were 1-20, 1-50 and 1-100 respectively. No agglutinins for S. suipestifer appeared in the serum of pig 113.

For the anemic group, the highest dilutions in which agglutinins for S. suipestifer were shown, ranged from 1-10 to 1-500. In ten of the twelve cases the dilutions were from 1-100 to 1-500. For the controls the range for highest serum dilutions was 1-20 to 1-200.

Figures 1 and 2 on the whole would seem to indicate that titers for S. aertrycke serum with S. aertrycke antigen generally ran higher in anemic than non-anemic pigs, also that for both groups of pigs titers for S. aertrycke were somewhat higher than for S. suipestifer when S.

aertrycke antigen was used.

2. Results of Tests on 20 Pigs Fed S. enteritidis. Table III shows these results. Fifteen anemic and five non-anemic pigs were in the group fed S. enteritidis. Six of the anemic pigs, however, are not included in comparisons. One of these, pig 69, showed no indication of agglutinin formation at any time, and was killed on the 19th day after feeding. Five other pigs, fed culture 332, and three fed culture 550, died 18 to 24 hours after feeding.

These five pigs together with pig 9 of the aertrycke group, died in all probability as a result of hog "flu". Several other pigs suffered from this disease, as indicated by their condition at autopsy. The combination of hog "flu" and the feeding of these organisms was probably the cause of death so soon after receiving the cultures. Under ordinary conditions, S. enteritidis appears to be non-pathogenic for pigs.

Culture 332 was discarded during the early part of the experiment on account of its self agglutinating properties. However, the first four pigs listed in Table III received this organism. Agglutinins are recorded as negative on the day of feeding because sufficient serum was

obtained from each pig and held until the next day, when it was retested with antigen made from culture 550. The findings as shown in Table III are the results of using antigen 550.

The groups for which comparisons are made include nine anemic pigs (2, 17, 57, 59, 60, 61, 63, 64 and 65), and five non-anemic controls. Here also, as in the S. aertrycke group, anemic pigs showed agglutinins earlier than non-anemic pigs. In the nine anemic pigs, agglutinins for S. enteritidis appeared in all cases by the sixth day after feeding, five of the nine appearing by the fourth day. Of the five non-anemic controls, none showed agglutinins by the sixth day, the first appearance in all cases being on the eighth day.

There was no cross agglutination between S. enteritidis and S. aertrycke, S. suipestifer, or E. coli. Antigens for these three organisms were used against each sample of serum collected from the enteritidis group of pigs, but no agglutinins appeared.

Figure 3 shows the first serum dilutions in which agglutinins appeared and the highest serum dilutions for the appearance of agglutination with S. enteritidis antigen. In the group of pigs fed S. enteritidis, the sera of

the nine anemic pigs which showed agglutination, gave a range of dilutions, when the first positive tests appeared, of 1-20 to 1-200. Highest serum dilutions, in which agglutination occurred in the anemic pigs, show a distinctly higher range, eight of the nine cases being from 1-100 to 1-500.

First dilutions in which agglutination appeared and highest dilutions in which agglutination was present are seen to be greater in the anemic than the non-anemic pigs. The range in the non-anemic pigs for the first serum dilutions where agglutination was present was from 1-20 to 1-100 and for the highest dilutions, 1-50 to 1-100.

3. Results of Tests on 26 Pigs Fed S. suipestifer. Nineteen anemic and seven non-anemic pigs which were fed S. suipestifer are listed in Table IV. Five anemic pigs and one non-anemic pig were dropped from the final comparisons. Two (25 and 26) of the five anemic pigs just mentioned were allowed to remain with their litter mates but received no cultures. Three other anemic pigs (15, 54, and 67) and one non-anemic pig (126) died soon after the feeding of cultures and before agglutinins appeared.

Final groups for comparison include therefore 14 anemic pigs and six non-anemic. Of the 14 anemic pigs,

12 showed agglutinins for S. suipestifer by the sixth day after feeding. In the six non-anemic controls, all cases showed these agglutinins by the sixth day and in four of the six, appearance was on the fourth day. The difference in time of appearance of agglutinins for the two groups does not seem significant.

Cross agglutination is not quite so evident here as in the group of pigs fed S. aertrycke. Agglutinins for S. aertrycke appeared in only six of the 14 anemic pigs; on thesecond, third, eighth, tenth (two cases) and twenty-second days respectively. In the six non-anemic pigs, cross agglutination is more evident, agglutinins for S. aertrycke appearing in all cases by the eighth day after culture feeding.

Figure 4 shows the first serum dilutions in which agglutinins appeared and the highest serum dilutions for the appearance of agglutinins of S. suipestifer. Much more variation is shown here than with the groups fed S. aertrycke and S. enteritidis. Comparisons for this group are probably no so valuable, as it will be seen from Table IV that the majority of the anemic pigs died in a few days after feeding of the organism.

For the 14 anemic pigs the titer range in the first dilution levels is 1-10 to 1-200, while the titer range

for the highest dilutions is 1-10 to 1-2000. The non-anemic control pigs fed S. suipestifer are of interest in that for them the highest serum dilutions showing agglutination of S. suipestifer range as follows: Two at 1-200; three at 1-500; and one at 1-10,000.

Figure 5 shows the first serum dilution points and the highest serum dilution points at which agglutination appeared for S. aertrycke, the minor agglutinin, in the groups of pigs fed S. suipestifer.

As has been noted, eight of the 14 anemic pigs failed to show these agglutinins for S. aertrycke. In the six showing positive tests the range of serum dilutions at which agglutinins first appeared is from 1-50 to 1-200, and for the highest dilutions the range is from 1-100 to 1-1000. High dilution points of the non-anemic control group present an interesting range, two at 1-100; one at 1-200; two at 1-500; and one at 1-10,000.

Pig 25 and 26, noted before as receiving no cultures but remaining with their litter mates, are of interest. They developed agglutinins for S. suipestifer, which probably indicates that although they picked up enough organisms to cause this reaction, still not enough organisms were present to produce lesions. Pig 25 developed

agglutinins for S. suipestifer on the sixteenth day, while pig 26 showed them for both S. suipestifer and S. aertrycke on the tenth day.

Pig 30 was also an interesting case. On the day it was fed the culture its serum was negative for any agglutinins. It was also negative on the second day after feeding. Death occurred some time during the third night. Serum was collected after death from the heart of this animal and subjected to the usual tests. Agglutination for S. suipestifer was complete up to and including the 1-100 dilution, and partial in the 1-200 dilution; while agglutinins for S. aertrycke were complete up to the 1-50 dilution, and partial in the 1-100 dilution.

Generally speaking, the results of S. suipestifer feeding correspond to the results obtained by other investigators. Biester, Murray, McNutt and Purwin (2) have recently dealt at length with S. suipestifer as the primary cause of the characteristic lesions produced when this organism is fed. The results obtained here agree with theirs in regard to the lesions found, also that the condition of the individual at the time of inoculation seems to have some bearing on the outcome.

4. Results of Tests on Seven Pigs Fed E. coli.

The results of these tests are recorded in Table V. It

will be remembered that only anemic pigs were used in this group, as non-anemic pigs were not available when this organism was fed. Agglutinins were present in a dilution of 1-20 in only one pig before feeding. Four other pigs produced agglutinins for this organism on the third day, while the remaining two did not develop any. One pig died on the sixth day and the other pig was killed on the same day. In all cases the agglutinins were specific for E. coli only. With the results obtained in this group, it is hardly justifiable to attach any particular significance to this organism. Partial agglutination in 1-100 in a few cases was the highest titer reached.

B. Bacteriological Examination.

In the description of the bacteriological examinations which were part of the experimental work, it was stated that the heart, liver and spleen of the 70 pigs used in the feeding experiments were cultured. Of the total number, 29, or 41.4 percent of the cases, yielded organisms when cultured. In these twenty-nine cases, the liver of pig 17 and the heart of pig 40 were the only organs that were negative. All other cultures revealed the various organisms in a pure state. Sub-cultures were made from these organisms for the study of the bacteriological and

morphological characteristics which are listed in Table VI.

It will be seen that in the characteristic traits of the genus Salmonella, the recovered organisms and the stock cultures that were fed show close correlation. Both groups of organisms are shown as gram negative, motile rods forming acid and gas in dextrose but not lactose. All showed inability to liquefy gelatin and were unable to produce indol.

While the above results are quite consistent, there might still be some doubt as to whether the organisms isolated were in reality the same strains that were fed, as there is still some controversy among workers regarding the identification of members of the colon-typhoid-enteritidis group on cultural characteristics alone. However, most of the investigators agree on several salient characteristics. Jordan (18) failed to get S. suipestifer to ferment arabinose while it fermented dulcite little or not at all. This agrees with the work of Krumwiede, Kohn and Valentine (19), Winslow, Kligler and Rothberg (20), Doyle and Spray (21), Spray (22) and others.

It will be noted that results with organisms re-

covered from pigs fed S. suipestifer 278 and 290 agreed on these two points, failure to ferment arabinose and also dulcite. This would seem to offer further proof of identification.

Jordan and Victorson (23) made use of lead acetate agar in differentiating S. suipestifer from S. enteritidis. They found that the former does not blacken this medium, while the latter does within 18 to 24 hours.

With one exception, results in the present tests checked on this point of differentiation. Organisms recovered from pigs fed S. enteritidis 332 and 550 showed blackening of lead acetate agar, while no blackening was noticed in any tests with organisms recovered from pigs fed S. suipestifer 278 and 290.

Although there is general agreement in the characteristics of the recovered organisms and the stock cultures, a few exceptions may be noted.

Cultures from pigs 11 and 40 failed to agree with S. aertrycke, the organism that was fed. They both failed to ferment arabinose and xylose with the production of acid and gas, and also produced a slight acidity in litmus milk which was followed by only a faint alkalinity. In these respects, they all fall into the classification of the S. sui-

pestifer group.

Pig 17 was fed S. enteritidis 332, but the organism recovered agreed with S. suipestifer in the same manner as cultures from pigs 11 and 40. The strains of S. enteritidis 332 and 550 that were fed produce higher acidity than the S. suipestifer strains and are followed by extreme alkalinity.

There was only one other slight variation, which was with the culture isolated from pig 1. It did not agree in all respects with S. suipestifer 290, the culture fed. In order to agree it should have produced acid and gas from xylose, while it produced only acid.

With these exceptions, all other organisms isolated agreed in all cultural and morphological characteristics with the stock cultures which were used in feeding.

C. Agglutinin Absorption Tests.

The agglutinin absorption tests, the results of which are recorded in Table VII, verify the results obtained from the study of cultural characteristics. For instance, pig 9 yielded a culture that apparently was the same as the one fed (S. aertrycke 179) and almost all the others showed corresponding results.

It will be noted that cultures from pigs 11 and 40 fall in the S. suipestifer group although S. aertrycke was fed. Cultures six, 16 and 17 were recovered from pigs which had received S. enteritidis 332. Organisms six and 16 were apparently the same as were fed on account of their behavior in these tests. They displayed self agglutinating properties as the control tubes in these tests were also positive. This condition, characteristic of S. enteritidis 332, aided in the identification of the organisms isolated from these two pigs. Culture 17 according to this test agrees with S. suipestifer.

All other organisms listed in this table check with the organisms which were fed.

D. Special Agglutination Tests.

The results of the first of these tests are recorded in Table VIII. They are a further check on the organisms isolated and verify the results which were found by all previous tests. The S. enteritidis (550) serum from pig 59 used in these tests caused agglutination in only seven sets of tubes. These included the five strains isolated from pigs fed S. enteritidis 550 and pigs 6 and

16 which have been previously described. Here again there was agglutination in the control tubes of the antigens made from these two organisms.

In a separate and final series of agglutination tests on 22 additional pigs, there were no agglutinins demonstrated for S. pullorum and E. sanguinarium. Tests were also made for agglutinins of the cultures used in feeding the main group of 70 pigs.

No agglutinins were shown in the sera of any of these pigs for S. aertrycke. Serum of pig 114 showed agglutination with the S. enteritidis antigen in the 1-10, 1-20 and 1-50 dilutions. The sera of pigs 89 and 118 showed agglutination in the 1-10 and 1-20 dilutions for S. suipestifer. Agglutination was also present in the 1-10 and 1-20 dilutions for E. coli when the sera of pigs 101, 102, 103, 119 and 138 were used.

The remaining 14 pigs of the group showed no agglutinins in their respective sera when tested against any of the various antigens.

The appearance of agglutinins to some extent in some of these pigs may possibly be attributed to the fact that, at four weeks of age, they were turned into outside lots where other pigs had been. They were here eight weeks,

and some of them may have picked up slight infections, sufficient to cause the production of antibodies.

E. General Discussion.

It should be noted, in a general consideration of results in this problem, that the pigs used as subjects received hog cholera anti-serum and in some cases virus also. Pigs 89 and 46 received serum at three weeks of age and an additional dose of serum and virus at 40 and 37 days of age respectively. Eighteen pigs (31, 32, 33, 34, 35, 42, 43, 44, 45, 48, 49, 57, 59, 60, 61, 63, 64 and 65) received serum and virus at approximately five weeks of age. The remaining pigs of the total group of 92 tested received only a single dose of serum at three weeks of age.

Very few of either anemic or non-anemic pigs showed agglutinins for any of the organisms studied before feeding of the cultures.

In the large group of 70 pigs, none of the 16 non-anemic controls showed agglutination for any of the organisms before feeding of the cultures. Only three of the 54 anemic pigs showed agglutinins before feeding. Pig 46 showed S. suipestifer agglutinins in its serum

in a 1-20 dilution before being fed S. aertrycke; pig 28 showed S. suipestifer agglutinins in its serum in a 1-100 dilution before the feeding of S. suipestifer; serum from pig 34 caused agglutination of E. coli antigen in a 1-20 dilution before feeding E. coli.

In the group of 22 extra pigs, sera of which were tested for agglutinins, only eight gave positive tests, one for S. enteritidis; two for S. suipestifer; and five for E. coli. All were in low dilutions. This would seem to be fairly good evidence that the amounts of serum and virus used in ordinary vaccination do not stimulate the production of antibodies in pigs.

Since the large majority of the pigs examined failed to show agglutinins in their sera before feeding, it would seem to indicate that these animals were not harboring the organisms at the time the first samples were taken. This observation correlates with certain findings of Jordan (24). He cultured the lower intestinal tract of 291 pigs and obtained 1419 strains of which only 40 were dextrose positive, lactose negative, and unable to liquefy gelatin. In his words, "the occurrence of true suipestifer strains in any abundance in the intestines of normal swine in this country is a rarity."

The results obtained in the present problem fail in some respects to agree with the work of Giltner (10) (11). He states that the blood of normal pigs agglutinates virulent cultures of B. cholera suis (S. suipestifer) in dilutions as high as 1-250, but usually less, and that serum of pigs having hog cholera as a result of virus inoculation may agglutinate as high as 1-800. The pigs that were treated by the serum-simultaneous method according to this investigator may agglutinate as high as 1-500.

Wehrbein (13) tested only eight normal pigs and found agglutinins for S. suipestifer in two pigs in dilutions of 1-10 and in one pig in 1-40. This percentage is higher than that found in the present work as only four of the total group of 92 pigs, both anemic and non-anemic, showed the presence of agglutinins for S. suipestifer in the first tests of the sera.

In every case in both the agglutination and the agglutinin absorption tests in which there was agglutination, the organisms accumulated in the bottom of the tubes in a floccular manner. The physical appearance agreed closely with the description given by Weil and Felix, as quoted by Savage and White (25).

V. SUMMARY AND CONCLUSIONS.

1. Three pigs, or 4.3 percent of the total group of 70, showed agglutinins before feeding of cultures. These three pigs (28, 46 and 34) were anemic. In the additional group of 22 pigs, the sera of eight showed agglutinins. Five of these were for E. coli, a normal inhabitant of the intestinal tract; one for E. enteritidis; and two for S. suipestifer. The presence of agglutinins of various members of the colon-typhoid-enteritidis group would seem, therefore, comparatively rare in young pigs.

2. Agglutinins for S. aertrycke and S. enteritidis appear earlier after feeding of cultures in anemic than non-anemic pigs. The earlier appearance of these agglutinins in anemic pigs is probably due to the ability of the organisms to penetrate the intestinal mucosa as a result of lowered resistance following anemia. Anemic pigs, in general, gave positive agglutination tests in higher serum dilutions than non-anemic pigs after feeding of both S. aertrycke and S. enteritidis.

3. Agglutinins for S. suipestifer appear in anemic pigs on an average in 5.2 days after feeding and in non-

anemic pigs in 6.0 days. Anemic pigs succumb more readily to artificial infection by S. suipestifer than non-anemic pigs. Of 19 anemic pigs, 11 died by the sixth day after feeding, as against one out of seven non-anemic pigs. Cohabitation of pigs not fed cultures with pigs fed cultures of S. suipestifer results in infection sufficient for stimulation of antibody production but insufficient for production of pathological lesions (pigs 25 and 26).

4. Agglutinins for E. coli, after feeding of the cultures, appeared in the anemic pigs but only comparatively low concentrations.

5. Anti-hog cholera serum and hog cholera virus, in amounts used in ordinary vaccination, do not stimulate the production of antibodies for the organisms studied.

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