Precipitation reactions in agar between swine serum and homologous pancreas extracts

by E. C. Pirtle*

SUMMARY

Hyperimmune anti-hog cholera and nonimmune swine sera yielded approximately 50% more precipitation reactions in agar-gel diffusion tests with pancreas extracts from SPF noninfected swine than with extracts obtained from swine experimentally infected with virulent hog cholera virus. The pancreas-reacting property of swine serum was determined to be relatively heat stable, withstanding 68 C for 30 minutes.

Of various swine serum fractions tested, the only one that reacted with pancreas extracts contained gamma, beta and alpha-globulins. In the absence of alpha-globulin, precipitation reactions were not observed.

Sera of newborn SPF piglets, containing 50% alpha-2 globulin, formed more intense precipitation lines with swine pancreas extracts than were formed by the sera of their dams with the same extracts.

The pancreas-reacting activity of swine sera was completely removed by absorption with pancreatic tissue. This property was not removed by absorption with guinea pig kidney, or beef, swine or human erythrocytes.

Maceration of pancreatic tissue released reactive substances in a polydispersed form. This was demonstrated by the ability of almost all supernates and sediments from differential centrifugation of such preparations to form precipitation lines with swine sera. Reactive substances from swine pancreas were found to be relatively heat labile, being inactivated in one hour at 56C.

No evidence was obtained in this study to indicate that the observed precipitation reactions were related to hog cholera virus and its corresponding antibody. The reactions are believed to have resulted from the interaction of protein-related substances present in normal swine pancreas with a relatively heat stable component, possibly alpha-globulin, in swine serum.

*From Virological Investigations, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

Introduction

The agar-gel double diffusion test (AGDT) has received much attention since 1954 as a possible aid in the laboratory diagnosis of hog cholera (swine fever), but reports regarding its value are controversial.

Using blood, lymph nodes or crystal violet vaccine prepared from blood of swine fever-infected swine as antigens, Molnar[1,2] observed single precipitation zones in agar when these materials were reacted with swine fever-immune serum. In one instance he observed two precipitation lines with infected lymph node material but did not indicate their significance. Precipitation lines were not observed when immune serum was reacted with blood or lymph nodes from uninfected swine.

In studying the applicability of the AGDT for several viruses and their homologous antisera, Mansi[3] concluded that swine fever-infected pancreas gave the best precipitation lines with homologous antiserum. Two precipitation lines were obtained between swine fever pancreas and antiserum, but no reactions were observed with control antigens or sera. When precipitation reactions were observed they were interpreted as being specific.

Forsek[4] examined pancreatic tissue in AGDT from pigs and rabbits experimentally infected with virulent and attenuated swine fever viruses, as well as from naturally infected pigs. Although it was not possible to distinguish between attenuated and virulent strains of virus the test was considered to be specific and useful for the detection of virus and the demonstration of immunity in vaccinated pigs. Using infected blood and organ extracts, Grishenkova[5] reported that he observed one sharp line of precipitate when his antigens were reacted with swine fever-immune serum, but none with control antigens.

Kulesko and Sobko[6] tested 183 pancreases from pigs experimentally infected with the Chinese "shi-min" strain of hog cholera

virus and 129 from healthy slaughtered pigs with hyperimmune rabbit anti-hog cholera serum in AGDT. They found that 95% of pancreases from the infected pigs gave positive precipitation reactions and approximately 25% of pancreases from the healthy pigs also gave positive results. When serum from infected pigs was used as antigen instead of pancreas, two precipitation lines formed in reaction with antiserum. Only one line was observed when anti-serum was reacted with serum from healthy pigs. The appearance of the second precipitation line with infected serum antigen was interpreted as indicating swine fever.

Pleva and Jurcina[7] also employed hyperimmune rabbit anti-swine fever serum in AGDT with swine fever-infected tissues. They concluded that mesenteric lymph nodes from pigs dead of swine fever gave the clearest precipitation lines, followed by pancreas, spleen and liver.

Janowski and Truszczynski[8] and Janowski[9] reported their results regarding the AGDT and its application to the detection of swine fever virus. It should be pointed out that the information presented in the first of the above two references is duplicated as a part of the second, and that there are discrepancies in the tabular data between the two.

In all of their experiments,[8,9] when normal and swine fever pancreas antigens were tested against normal and positive swine sera in the same agar-gel plates, a continuous, fused precipitation band was produced. They likewise obtained an equivocal frequency (nearly 100%) of precipitation lines when normal and swine fever pancreas antigens were reacted with either normal or hyperimmune rabbit anti-swine fever serum. These workers attributed their results, in part, to the presence in normal and swine fever-immune swine and rabbit sera of physiological "anti-pancreas" antibodies. Although they did not attempt absorbing the "anti-pancreas" antibodies from the sera, they significantly reduced the serological activity of pig sera by heating them at 62 C for 30 minutes before testing with pancreas antigens. Rabbit sera heated at 56 C for 30 minutes did not show a reduced reactivity with pancreas antigens.

Darbyshire[10,11,12] employing the AGDT, reported on a serologic relationship between a mucosal disease and swine fever. The swine fever antigens[12] were prepared from pancreases of experimental pigs dead of swine fever and were chosen as test antigens on the basis of giving a precipitation line with swine fever-immune or hyperimmune serum. Of 50 swine fever pancreas antigens tested, it was not stated how many gave precipitation lines with homologous antiserum, only that the reactivity was identical (positive or negative) with both swine fever and mucosal disease antisera. Control sera and antigens employed in the agar-gel tests were not observed to react. The serologic activity of the two types of antiserum was removed by prior absorption with either homologous or heterologous test antigens. Similar prior absorption with normal control tissues did not alter the serologic activity of the two types of antiserum. The results of the absorption studies indicated to Darbyshire that the development of precipitation lines and reactions of identity in the test systems involved a true antigen-antibody reaction.

Keast, *et al.*[13] undertook to evaluate the AGDT as a diagnostic aid in swine fever of low virulence. In their laboratory, interpretation of the test was based on the capacity of an unknown serum or pancreas antigen to deflect a precipitation reference line formed by reacting specific swine fever antigen and homologous antibody. Reactions encountered with unknown pancreas specimens were classified as negative, suspicious, weak positive, positive, non-optimal proportions, and non-specific. Antigen was detected in pancreases of approximately 25% of swine dead of virulent virus infection and it was estimated that not more than 5% of swine infected with virulent virus would yield satisfactory reference antigens.

A critical evaluation of the AGDT (using pancreatic tissue as antigen) in the diagnosis of hog cholera, its specificity, sensitivity and reliability, was initiated in this laboratory in 1962. Early in these studies it was found that hog cholera-immune swine serum formed precipitation lines in agar-gel when reacted with many preparations of pancreas from swine experimentally infected with virulent hog cholera virus, as well as with an outstanding number of pancreas preparations from normal swine. It was further found that a signal number of normal swine sera formed precipitation lines when reacted with either

normal or hog cholera pancreas preparations. Precipitation lines were variable in number, sharpness and intensity. In view of the frequency of precipitation reactions of infected and noninfected tissues with both immune and nonimmune serum, this study was carried out in an attempt to elucidate the basis for these reactions.

Materials and Methods

Agar-gel double diffusion tests (AGDT). — The agar formula employed in this study contained 0.75% purified agar* and 0.01% Merthiolate** dissolved in freshly distilled water. After autoclaving at 15 lbs. pressure for 15 minutes, 10 ml amounts of melted agar were pipetted into petri dishes 50 mm in diameter and 10 mm deep. Wells were cut in solidified agar with cork borers using appropriate templates. Agar plates had a six or seven-well pattern, a central well and either five or six outer wells. The wells were 6 mm in diameter and were 9 mm apart from their outer edges. Approximately 0.2 ml of serum or pancreas extract was delivered into each well. AGDT were incubated at room temperature, approximately 22 C, and observed daily for appearance of precipitation lines. Precipitation lines generally appeared between 48 to 72 hours, but all plates were held for at least 10 days before final recordings were made.

Experimental animals and tissues. — Hog cholera susceptible swine, 4-12 months of age, were obtained through the animal quarantine section (A.Q.) of this laboratory. These swine were experimentally infected by inoculation with the National Animal Disease Laboratory strain of virulent hog cholera virus (HCV). Pancreatic tissue (HC-Pan) was obtained from them following exsanguination when moribund or very shortly after death, 6-11 days after injection. For control tissues, pancreases were obtained from 53 specific pathogen-free swine (SPF-Pan) ranging in age from 6 weeks to 4 months. The blood types of 12 of these were known. Tissues were sealed in plastic bags and stored at -20 C or -70 C until used. Both of these temperatures proved satisfactory for storage of tissues during this study.

Preparation of tissues for testing. — Twenty-five percent suspensions (w/v) of HC-Pan and SPF-Pan were prepared by grinding each tissue, mixed with sand, with a sterile mortar and pestle. They were suspended in 0.01 M phosphate buffered saline (PBS), pH 7.2, containing Merthiolate 1:10,000. Excess adipose tissue was removed prior to grinding. After allowing the gross particulate matter to settle, the extracts were employed as crude "antigen" in AGDT. In some experiments crude extracts were subjected to fractional centrifugation for 30-minute intervals in an International PR-2* refrigerated centrifuge and a Spinco** Model L ultracentrifuge, at forces ranging from 980 x g to 105,400 x g. Supernatant fluids, and sediments resuspended to original volume in PBS, were used as antigens in AGDT. In other experiments clarified pancreas extracts (980 x g, 30 minutes) were heated at various temperatures for a 30-minute period prior to testing.

SERUM AND SERUM FRACTIONS.

1. Hyperimmune anti-hog cholera swine serum (HHCS). The three sera employed were produced in this laboratory.[14] They are designated S-2876(30), S-2880(304) and S-9197. We are indebted to Dr. A. W. McClurkin for S-2876(30) and to Dr. J. P. Torrey for S-2880(304) and S-9197.

2. Nonimmune SPF swine serum (NISS). Sixty sera from young and adult swine were obtained through the A. Q. section. Some of these sera were pooled after their individual reactivities had been determined.

3. Sera from SPF swine of known blood types. We are indebted to Dr. Eric Andresen of Iowa State University for performing blood typing tests on 12 SPF, nonimmune swine. Blood types were classified as A, or O or i.[15]

4. Sera from newborn SPF swine and their dams. Forty-eight newborn piglets were bled immediately after being delivered from 16 dams by hysterectomy. Serum was also obtained from each dam. An electrophoretic study of the piglet sera[16] yielded the following mean values: alpha-2 globulin, 51.6%, alpha-1 globulin, 24.2%, beta-globulin, 13.3%, albumin, 5.9% and gamma-globulin, 5.0%. Likewise,[16] the following mean values were obtained for the sera from the dams: alpha-globulin, 19.4%, be-

*Difco, Inc., Detroit, Michigan.
**Eli Lilly, Indianapolis, Indiana.

*International Equipment Co., Boston, Massachusetts.
**Spinco Division, Beckman Instruments, Palo Alto, California.

ta-globulin, 14.8%, albumin, 46.8% and gamma-globulin, 19.0%.

5. Serum fractions. Gamma-globulin was prepared by $(NH_4)_2SO_4$ fractionation of HHCS, S-2880(304) and S-9197 and a pool of two NISS according to the method of Kendall.[17,18] Electrophoretically-pure gamma-globulin from NISS was designated NISS-G-1. Two additional globulin fractions, designated NISS-G-2 and NISS-G-3, were prepared from the same NISS pool by initial precipitation with one-half saturation with $(NH_4)_2SO_4$, followed by reprecipitations with lesser concentrations of $(NH_4)_2SO_4$. By electrophoresis, NISS-G-2 contained 58.7% gamma and 41.3% beta-globulin; NISS-G-3 contained 51.5% gamma, 23.5% beta and 25.0% alpha-globulin. Electrophoretically-pure porcine gamma-globulin and albumin were also obtained from a commercial source.*

Sera were used either unheated or heated at 56 C for 30 minutes. In one experiment, designed to determine the effect of heating on the reactivity of serum with pancreas extracts, sera were heated for 30-minute intervals at various temperatures.

Absorption of sera. — HHCS and NISS, which formed good precipitation lines with pancreas extracts when diluted 1:5 in PBS, were chosen for absorption experiments.

1. To determine the possibility of the presence of heterophilic antibodies, sera were absorbed with guinea pig kidney and boiled beef erythrocytes according to Davidsohn, *et al.*[19] except that absorptions were carried out twice on each serum.

2. To determine whether there was a correlation between the observed precipitation reactions and certain blood group antigens, sera were absorbed with erythrocytes (RBC) from type A swine[15] and types A (Rh-), B (Rh+) and O (Rh+) from human donors. Sera were diluted 1:5 in PBS and 10.0 ml added to 1.0 ml of three-times washed, packed RBC of each type. Absorptions were carried out at 4 C overnight. Sera were absorbed twice with each RBC type before being retested with pancreas extracts with which precipitation lines were produced prior to absorption.

3. To determine the ability of pancreatic tissue to remove pancreas-precipitins from hyperimmune and nonimmune sera, these sera were absorbed with pancreatic tissues from nonimmune SPF swine whose blood types were known (A and O or i) and from comparable swine whose blood types were not known. Sera, diluted 1:5, were absorbed once with 2 gm (wet weight) of finely minced pancreas added to 10 ml of serum. Absorption was carried out at 4 C in 125-ml flasks which were agitated several times during a 48-hour period. Initial clarification of absorbed serum was carried out at 3,000 rpm for 15 minutes in the refrigerated centrifuge and final clarification at 30,000 rpm for one hour in the Model L Spinco (40 rotor). Unabsorbed portions of the same sera were likewise centrifuged. Pancreas-absorbed and unabsorbed sera were then retested in AGDT with pancreas extracts with which they had initially produced precipitation lines. Absorbed and unabsorbed sera were also subjected to electrophoresis.

Electrophoresis of swine serum. — A Beckman* Model R electrophoresis system was employed. A veronal buffer at pH 8.6, ionic strength 0.075, was used in the cell. The quantity of material applied to each paper strip was 0.006 ml. Sera and serum fractions were separated at 2.5 milliamperes constant current for 16 hours. Duplicate runs were made on all materials. Strips were stained with 0.1% bromphenol blue dye in absolute methanol. The strips were scanned and evaluated with a Spinco, Model RB Analytrol calibrated for serum protein (Procedure B).

Results

Reactions of HHCS and NISS with pancreas extracts. — Three HHCS and 72 NISS were tested in numerous AGDT employing 73 HC-Pan and 53 SPF-Pan crude extracts. Of 642 tests performed (Table 1) a remarkably greater incidence of precipitation lines was observed in reactions between HHCS and NISS with SPF-Pan extracts than with HC-Pan extracts. From one to four precipitation lines were observed in the various tests performed, but it was not possible to demonstrate any correlation between the number of precipitation lines and any given pancreas-serum reaction. Furthermore, when precipitation lines were observed between HHCS and a given pancreas extract, precipitation lines were always also observed between the same extract and NISS. It was observed

*Nutritional Biochemicals Corp., Cleveland, Ohio.

*Spinco Division, Beckman Instruments, Palo Alto, California.

TABLE 1 — Reactions of Hyperimmune Anti-Hog Cholera (HHCS) and Nonimmune (NISS) Swine Sera with Swine Pancreas Extracts

Serum	Pancreas Extracts: HC-Pan Number of Tests	HC-Pan Number Positive*	HC-Pan Percent Positive	SPF-Pan Number of Tests	SPF-Pan Number Positive*	SPF-Pan Percent Positive
HHCS	256	141	55	74	70	94
NISS	67	38	57	245	236	96

*Precipitation lines observed.

TABLE 2 — Reactions of Swine Serum Globulins and Albumin with Pancreas Extracts

Serum Fraction	Pancreas Extracts: HC-Pan	SPF-Pan
S-2880(304) gamma-globulin	0/10*	0/18
S-9197 gamma-globulin	0/10	0/18
Commercial gamma-globulin	0/10	0/18
NISS pool	4/10	9/10
NISS-G-1	0/10	0/18
NISS-G-2	—**	0/6
NISS-G-3	—	6/6
Commercial albumin	0/14	1/42
NISS-G-1 + albumin	0/14	0/12
NISS-G-2 + albumin	0/14	0/12

**Numerator, number of precipitation reactions observed. Denominator, number of tests performed.
**Not tested.

that a NISS produced precipitation lines as readily with a pancreas extract from the same animal from which the serum was obtained as with other SPF-Pan extracts. When undiluted serum was used in AGDT precipitation lines usually formed very close to the wells containing pancreas extract. However, when serum was diluted 1:5 in PBS, lines formed approximately equidistant from serum and extract wells.

Reactions of swine serum fractions. — Four gamma-globulin preparations, two from HHCS, one from a NISS pool and one commercial swine serum gamma-globulin were dissolved in PBS in a concentration equivalent to that reported for adult swine serum.[16,20]. A commercial swine serum albumin solution was prepared on the same basis. In addition to being tested alone, mixtures of equal volumes of NISS-G-1 and NISS-G-2 with swine albumin were prepared from separate solutions containing approximately twice the reported mean values. These nine preparations were reacted in AGDT with HC-Pan and SPF-Pan extracts known to have produced precipitation lines with individlal HHCS and NISS (Table 2). Gamma-globulins and albumin, alone or in combination, and NISS-G-2 composed of gamma and beta-globulins did not visibly react (one exception) with pancreas extracts. However, NISS-G-3 composed of gamma, beta and alpha-globulins reacted as readily in AGDT with SPF-Pan extracts as the NISS from which it was prepared.

Reactions of sera from newborn SPF piglets and their dams with SPF-Pan extracts. — The sera of 48 SPF piglets and their 16 dams were reacted in AGDT with a pool of three SPF-Pan extracts which had produced individual precipitation reactions with 30 other HHCS and NISS. All 64 sera produced precipitation lines with the SPF-Pan pool extract. The intensity of the precipitation lines with piglet sera was more outstanding than those obtained with sera from the dams.

Reactions of heated NISS. — A pool of two NISS which had previously formed precipitation lines with several HC-Pan and SPF-Pan extracts were heated at temperatures ranging from 37 C to 68 C for 30 minutes and tested in AGDT with a pool of three SPF-Pan extracts with which they had produced individual reactions (Table 3). The reactivity of the various samples was equivocal even though the

electrophoretic pattern of the serum changed dramatically with increases in temperature. As the temperature increased the faster moving components decreased in concentration and became progressively incorporated into the slower migrating components. At 68 C approximately one-half of the total serum components were precipitated and did not migrate during electrophoresis.

TABLE 3 — Reactions of Heated Nonimmune Swine Serum (NISS) with SPF Pancreas (SPF-Pan) Extract

Serum	Treatment	Reactivity with SPF-Pan
NISS pool	Heated 37 C, 30 minutes	+*
	Heated 56 C, 30 minutes	+
	Heated 64 C, 30 minutes	+
	Heated 66 C, 30 minutes	+
	Heated 68 C, 30 minutes	+

*Precipitation lines observed.

TABLE 4 — Reactions of Absorbed Swine Sera with Swine Pancreas Extracts

Serum	Absorbed With	Reactivity With HC-Pan	Reactivity With SPF-Pan
HHCS S-2880 (304)	None	+*	+
	G. P. Kidney	+	+
	Beef RBC	+	+
	Swine A RBC	+	+
	Human A RBC	+	+
	Human O RBC	+	+
	Human B RBC	+	+
	SPF-Pan, type A animal	−**	−
	SPF-Pan, type O animal	−	−
	SPF-Pan, type not known	−	−
NISS (5 individual sera)	None	+	+
	G. P. Kidney	+	+
	Beef RBC	+	+
	Swine A RBC	+	+
	Human A RBC	+	+
	Human O RBC	+	+
	Human B RBC	+	+
	SPF-Pan, type A animal	−	−
	SPF-Pan, type O animal	−	−
	SPF-Pan, type not known	−	−

*Precipitation lines observed.
**No precipitation lines observed.

Reactions of absorbed swine sera. — One HHCS and five individual NISS were absorbed with guinea pig kidney, beef, swine and human erythrocytes (RBC) and also with SPF-Pan tissue (Table 4). In all instances absorption of the six sera with SPF-Pan tissues removed all HC-Pan and SPF-Pan reacting activity, whereas the other materials employed did not absorb pancreas precipitins from these same sera. Electrophoretic analysis of the pancreas-absorbed sera indicated a reduction of all serum components.

Reactivity of centrifuged pancreas extract. — An SPF-Pan extract which formed precipitation lines in reaction with both HHCS and NISS was subjected to fractional centrifugation. The resulting supernatant fluids and resuspended sediments were tested in AGDT with the same HHCS and NISS that reacted with the original extract (Table 5). Supernatant fluids from all centrifugations reacted with both sera. The clearest and most intense lines were obtained with supernates from 980 x g and 6,590 x g. The other three supernates formed observable lines of lesser intensity. The sediments derived from the lowest and highest centrifugal speeds did not form precipitation lines with the two sera; the sediments derived from the three intermediate speeds did. Tissue debris, represented by the sediment collected at the lowest centrifugal speed (980 x g), was apparently depleted of pancreas reactive substances during grinding.

Reactivity of heated pancreas extracts. — Clarified (980 x g, 30 minutes) HC-Pan

TABLE 5 — Reactions of Hyperimmune Anti-Hog Cholera (HHCS) and Nonimmune (NISS) Swine Sera with Centrifuged SPF Pancreas (SPF-Pan) Extract

Centrifugation of Extract	Swine Sera HHCS	Swine Sera NISS
Crude extract	+*	+
980 x g Supernate	+	+
Sediment	−**	−
6,590 x g Supernate	+	+
Sediment	+	+
23,360 x g Supernate	+	+
Sediment	+	+
59,310 x g Supernate	+	+
Sediment	+	+
105,400 x g Supernate	+	+
Sediment	−	−

*Precipitation lines observed.
**No precipitation lines observed.

TABLE 6 — Reactions of Hyperimmune Anti-Hog Cholera (HHCS) and Nonimmune (NISS) Swine Sera with Heated Hog Cholera Pancreas (HC-Pan) and SPF Pancreas (SPF-Pan) Extracts

Serum	Heated Pancreas Extracts*			
	22 C, 30 min.	37 C, 30 min.	56 C, 30 min.	56 C, 1 hr.
HHCS	+**	+	±***	−****
NISS	+	+	±	−

*Identical results obtained with both extracts.
**Precipitation lines observed.
***Barely detectable lines observed.
****No precipitation lines observed.

and SPF-Pan extracts, after being heated at 37 C or 56 C, were tested in AGDT with HHCS and NISS (Table 6). Indentical results were obtained with both extracts. Extracts incubated at room temperature (22 C) and at 37 C produced observable precipitation lines with both sera within 24 hours. The extracts heated at 56 C for one hour did not form lines during the ten-day period of observation.

Discussion

Several workers have observed precipitation reactions in agar-gel which were not related to antigen-antibody reactions. These involved interactions of normal sera of various species with a soluble liver component,[21] rat tissue extracts,[22] and homogenates,[23] human organ extracts,[24] hemolysates of erythrocytes[25] and a bovine pancreatic enzyme.[26] Peetoom, *et al.*[25] and Deckers and Maisin[22] identified the serum substance producing nonimmune precipitation in their test systems as alpha-2 globulin.

During this study it was observed that HHCS and NISS produced precipitation reactions nearly twice as frequently with noninfected SPF swine pancreatic tissues as with pancreatic tissues from swine experimentally infected with hog cholera virus. On this basis alone it is difficult to assess any validity to the specificity of the AGDT in detecting hog cholera viral antigen in pancreatic tissue or corresponding serum antibody. The much fewer number of precipitation reactions with HC-Pan extracts possibly can be attributed to hog cholera infection bringing about partial destruction or alteration of tissue components normally present in swine pancreas.

In attempting to determine what portion of the serum complex was responsible for reactivity with pancreas extract it was found that swine gamma-globulin, NISS-G-2 (gamma and beta-globulins) and albumin serum fractions did not form precipitation lines in AGDT. If these fractions are considered to be representative of their individual potentialities as contained in native swine serum, it can be assumed that neither of them was active in the formation of precipitation lines when reacted with pancreas extracts. However, NISS-G-3, composed of gamma, beta and alpha-globulins was as reactive with SPF-Pan extracts as the NISS from which it was derived. This evidence suggests that alpha-globulin played an important role in observed precipitation reactions between swine serum and pancreas extracts.

Before the newborn SPF piglet sera were run in AGDT it was predicted that no precipitation lines would be formed with SPF-Pan extracts. However, more intense lines were formed with these sera than with adult swine sera and the same extracts. A possible explanation for this unexpected result perhaps is related to the fact that the piglet sera contained approximately 50% alpha-2 globulin, but relatively low levels of gamma-globulin and albumin, approximately 5.0% each. If pancreas reactivity of both adult and piglet serum is related to the content of alpha-globulin, the more intense reactions with piglet serum may be attributed to a relatively higher concentration of this serum component.

The pancreas-reacting principle in NISS is relatively heat stable, withstanding 68 C for 30 minutes. Although the electrophoretic pattern indicated that half of the serum components were precipitated, the 68 C sample still possessed pancreas precipitins. It is obvious that even though molecular alteration and precipitation of serum components occurred during heating, there was

not a simultaneous destruction of the pancreas-reacting principle.

Because of the failure of guinea pig kidney and beef erythrocytes to remove the pancreas-reacting activity from HHCS and NISS this property of swine serum does not appear related to heterophilic antibodies. Likewise, the reactive property does not appear related to certain blood group substances as demonstrated by its failure to be absorbed by swine or human erythrocytes. However, in dramatic contrast, pancreas precipitins were completely absorbed from both HHCS and NISS by pancreatic tissue. This phenomenon, as well as the formation of precipitation lines in AGDT, apparently resulted from the combination of substances present in normal pancreatic tissue with a component, possibly alpha-globulin, present in swine serum, and was not the result of a true antigen-antibody reaction. It is thus not surprising that electrophoresis of pancreas-absorbed serum failed to indicate an absolute reduction of any single serum component.

The reactivity of pancreas supernates and sediments derived from a wide range of differential centrifugation indicates that the reactive material was released from tissue in a polydispersed form. As a variety of pancreas extracts were observed to yield from one to four precipitation lines when reacted with a single serum, it is further suggested that some pancreases possess more than one potentially reactive substance.

The substances from swine pancreas which react with swine serum in AGDT are relatively heat labile, being partially altered at 56 C in 30 minutes and entirely inactivated at this same temperature in one hour. Although the reactive substances were not identified in this study the above evidence suggests that they may be related to protein.

Although no evidence was obtained during this study to indicate that the observed precipitation reactions were related to hog cholera virus and corresponding antibody, the AGDT may find application in this disease after a specific, relatively tissue-free, hog cholera antigen becomes available.

REFERENCES

1. **MOLNAR, I.**: Precipitation experiments with swine fever virus-containing material. Acta vet. Hung., **4**, (1954): 247-251.
2. **IBID.**: Precipitációs kísérletek sertéspestis virusát tartalmazó anyagokkal. Allat. Lapja, **9**, (1954): 146-148.
3. **MANSI, W.**: The study of some viruses by the plate gel diffusion precipitin test. J. Comp. Path. and Therm., **67**, (1957): 297-303.
4. **FORSEK, M.Z.**: Primjena gel-difuzionog precipitin-testa u dokazivanju virusa svinjske kuge. Vet. Glasnik., **12**, (1958): 867-871.
5. **GRISHENKOVA, A. S.**: Agar precipitation reaction for the laboratory diagnosis of swine fever. Proc. Lenin Acad. Agric. Sci., **23**, (1958): 35-37.
6. **KULESKO, I. I., and SOBKO, A. I.**: Diffusion reaction of precipitation on agar plate for hog cholera diagnostics. Vet. (Moscow), **37**, (1960): 68-73.
7. **PLEVA, J. and JURCINA, A.**: Diagnostika moru osípaných pomocou precipitácie v agare. Vet. Cas., **10**, (1961): 117-121.
8. **JANOWSKI, H. and TRUSZCZYNSKI, M.**: Serological studies on swine fever: I. The gel-diffusion precipitin test. Pulway (Poland) Inst. Weter. Biul., **3**, (1959): 6-13.
9. **JANOWSKI, H.**: Some studies on swine fever. Off. Internat. des Epiz. Bull., **56**, (1961): 273-298.
10. **DARBYSHIRE, J. H.**: A serological relationship between swine fever and mucosal disease of cattle. Vet. Rec., **72**, (1960): 331.
11. **IBID.**: Agar gel diffusion studies with a mucosal disease of cattle. I. Preliminary results with the technique. Res. Vet. Sci., **3**, (1962): 118-124.
12. **IBID.**: II. A serological relationship between a mucosal disease and swine fever. Res. Vt. Sci., **3**, (1962): 125-128.
13. **KEAST, J. C., LITTLEJOHNS, I. R. and HELWIG, D. M.**: Experiences in the laboratory diagnosis in swine fever of low virulence. Austral. Vet. J., **38**, (1962): 129-137.
14. **CODE OF FEDERAL REGULATIONS**, Title 9, Chapter 1, part 119, (1959).
15. **ANDRESEN, E.**: Blood groups in pigs. Ann. N. Y. Acad. Sci., **97**, (1962): 205-225.
16. **PIRTLE, E. C. and DEYOE, B. L.**: Electrophoresis of serum from specific pathogen-free (SPF) swine. Am. J. Vet. Res., **24**, (1963): 762-765.
17. **KENDALL, F. E.**: Studies on serum proteins. I. Identification of a single serum globulin by immunological means. Its distribution in the sera of normal individuals and of patients with cirrhosis of the liver and with chronic glomerulonephritis. J. Clin. Invest., **16**, (1937): 921-931.
18. **IBID.**: The use of immunochemical methods for the identification and determination of human serum proteins. Cold Spring Harbor Symp. Quant. Biol., **6**, (1938): 376-384.
19. **DAVIDSOHN, I., STERN, K. and KASHIWAGI, C.**: The differential test for infectious mononucleosis. Am. J. Clin. Path., **21**, (1951): 1101-1113.
20. **KNILL, L. M., PODLESKI, T. R. and CHILDS, W. A.**: Normal values of swine serum proteins. Proc. Soc. Exptl. Biol. and Med., **97**, 1958): 224-226.
21. **TOMASI, T. B.**: A precipitation reaction between human serum and a soluble tissue component. J. Immunol., **86**, (1961): 427-430.
22. **DECKERS, C. and MAISIN, J.**: Non-specific precipitation patterns obtained in double diffusion between serum and extracts of normal organs and tumors. Nature, **197**, (1963): 397.
23. **BERENBAUM, M. C., KITCH, G. M., and COPE, W. A.**: Spurious "auto-immune" reactions in gel-diffusion plates. Nature, **193**, (1962): 81-82.
24. **METZGAR, R. S. and GRACE, J. T.**: Agar precipitation reactions between human serum albumin and hemoglobin in human tissue extracts. J. Immunol., **86**, (1961): 578-583.
25. **PEETOOM, F., ROSE, N., RUDDY, S., MITCHELL, A. and GRABAR, P.**: Etude des hémolysats des globules rouges humains. II. Présence dans les sérums normaux d'une substance précipitant l'hémolysat. Ann. Inst. Pasteur, **98**, (1960), 252-260.
26. **NIECE, J. L. and BARRETT, J .T.**: Non-immune gel precipitin tests with an enzyme. Nature, **197**, (1963): 1021.