THE EFFECTS OF CANDIDA ALBICANS ON THE ESOPHAGOGASTRIC AREA OF THE PORCINE STOMACH

by

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INTRODUCTION

Gastric ulcers have long been known to occur in the glandular portion of the porcine stomach. Many times these ulcers accompanied diseases causing chronic gastroenteritis. Ulceration of the glandless esophageal portion was described by Bullard (1951). Since that time, esophagogastric ulcers have been reported with increasing frequency.

Losses from gastric ulcers have been reported at the various swine testing stations throughout the country. Presently, field cases are appearing in widely separated herds with regularity. In Kansas, losses have varied from one pig in a herd to 20% mortality rate within a five day period.

The cause of esophagogastric ulcers has been attributed to many entities. Various chemical elements and antibiotics used as feed additives have been incriminated. Environmental conditions and diet have been suggested as playing a role in the etiology. The yeast-like fungus, Candida albicans, has been considered to have a keratinolytic or keratinogenic effect on the esophagogastric epithelium. The etiology and the pathogenesis are, at best, incompletely understood.

This experiment was designed to study the effects of C. albicans on the esophagogastric area of the stomach. Mycologic findings were correlated with the gross and histopathologic data.

REVIEW OF LITERATURE

Bullard (1951) published the first specific article on esophagogastric ulcers. Upon necropsy of a 500 pound boar, the only lesion found

was a large round ulcer completely surrounding the esophageal orifice. The animal was anemic, and there was blood throughout the small and large intestine. Few succeeding references can be found on this subject until 1959. The possibility that a new condition had originated is probable. However, there is a remote possibility that this condition has gone unnoticed for some time.

An alimentary mycotic condition involving pigs on an excessive or prolonged intake of antibiotics was described by Quinn (1952). The area of involvement was from the esophagus to the large intestine. In one pig the cardia was occluded by an inflammatory fungal lesion which yielded pure cultures of Monilia (Candida). Losses dropped when antibiotics were withheld.

Kowalczyk (1958) reported 6 cases of stomach ulcers. Some pigs were housed in groups of 5 to 7, while others were in a group of 50 kept outdoors. A balanced ration was fed.

In Great Britain an outbreak of mucormycosis was observed by Gitter and Austwick (1959) in pigs that were two days to 13 days old. C. albicans was found concurrently with Rhizopus microsporus. It was noted that C. albicans only invaded the stratified squamous epithelium of the stomach, esophagus, mouth, and tongue. R. microsporus invaded only the glandular parts of the intestinal tract. Lesions attributed to candidiasis were characterized by an excessive amount of mucus and desquamated cells found on the surface of the epithelium. The stratified squamous epithelium showed degenerative changes from the invasion of yeast cells and pseudomycelia. Congestion of the blood vessels in the submucosa was also noted.

Kowalczyk et al. (1960) were able to demonstrate stomach ulcers by gastroscopic examination. Most were located in the esophagogastric area of the stomach. In early stages, small wart-like proliferations of the stratified squamous were observed. These areas would slough, leaving small, dry, rough areas. As the process continued, the small areas would coalesce with others. As the areas increased in size, they also became deeper. When erosion of an artery took place varying amounts of hemorrhage occurred. Kowalczyk et al. (1960) concluded that stomach ulcers were not uncommon in swine. Over a three year period, they recorded 12 deaths in 161 swine. There was no apparent relationship of gastric ulcers to dietary supplements of zinc, copper and cadmium. However, there was an indication that a seasonal factor was involved.

Osborne et al. (1960) observed a Candida infection in a group of 140 artificially reared pigs. Ninety-three lived to 8 weeks of age. Lesions of candidiasis were observed in 25 of the 47 that died. Yeast cells and pseudohyphae were mixed with desquamated epithelium and neutrophils. The organisms demonstrated were usually in the superficial layers of the epithelium. Occasionally the pseudohyphae would penetrate the mucosa, but the cellular reaction was the same as the areas that contained no pseudohyphae. It was suggested that a low-grade toxemia produced by Escherichia coli, strain OK57, may have been a predisposing factor. These workers also noted candidiasis was primarily present in pigs on a diet containing large amounts of sugar.

Esophagogastric ulcers occurred in swine at the boar testing stations in Iowa. Berg (1960) suggested that management practices, confinement,

and high energy-low fiber ration may have been the cause of these lesions.

In England, Buntain (1961) reported deaths due to esophagogastric ulcers in pigs on a nutritional experiment. Various trace elements were added to the diet in large quantities. The deaths were almost entirely limited to the groups fed large amounts of copper; however, these results could not be repeated. It was considered that copper plus an unknown toxic factor was the causative factor.

Thoonen and Hoorens (1961) in Belgium, found a 5% incidence of gastric ulcers in 600 pigs presented for post-mortem examination during a two-year period. Most ulcers were in the region of the cardia. Of the 29 ulcers observed, 10 were positive for Geotrichum or Candida. In all cases, lesions of other diseases were found. They found no relationship of "molds", feed supplements, or parakeratosis in gastric ulcers.

In two experiments Perry et al. (1963) produced esophagogastric ulcers by feeding galatinized corn (heat treated). In the first experiment, the ulcers were responsible for a 39% mortality rate. The pigs receiving gelatinized corn in the second experiment had a 53% incidence of ulcers, and the mortality rate was 11%. No ulcers were observed in control groups receiving raw yellow corn.

Griffing (1963) examined 610 pigs from six different sources. His results suggested that keratinization contributed to the pathogenesis of esophagogastric ulcers. Bacteriologic and mycologic studies revealed that only C. albicans could be isolated routinely from the ulcers or denuded epithelium.

C. albicans was isolated from eight herds in northwestern Wisconsin which were encountering heavy death losses. Baker and Cadman (1963)

isolated the organism from the esophageal region of the stomach. Ulcerative and inflammatory lesions were also reported in this region.

Rothenbacher et al. (1963) described cardiac ulcers in Michigan pigs. They found no relation of this type of ulcer to the peptic ulcer commonly associated with the glandular areas of the stomach. A relationship of the parakeratotic proliferative changes in the erosion and ulceration existed. These workers supported the theory that some toxic agent probably found in the feed was responsible for the pathogenesis of esophagogastric ulcers.

Swine from the Indiana Swine Evaluation Station were observed by Curtin et al. (1963). Of 443 animals observed, 87 showed evidence of ulcers. C. albicans was consistently isolated from the lesions. Pseudohyphae and yeast cells were often observed on histopathologic examination. Rapid growth of the pigs appeared to be important to the genesis of the esophagogastric ulcers. A seasonal occurrence also was noted.

Blaxland and Markson (1954) made some interesting observations on candidiasis in the turkey. Antibiotics in feed were thought to be a contributing factor. Malnutrition or other external factors were suspected of playing an important role in candidiasis. These authors felt that increased virulence of the fungus is a possibility that should not be overlooked.

The keratinolytic properties of C. albicans in the presence of glucose was demonstrated by Kapica and Blank (1957).

Numerous references indicate C. albicans is commonly isolated from esophagogastric ulcers. Further studies are warranted in order to

determine the specific role of C. albicans in the pathogenesis of esophagogastric ulcers.

MATERIALS AND METHODS

Survey Specimens

Collecting and Processing. Specimens were collected at a Kansas City, Kansas abattoir. For the first 30 minutes of each hour, stomachs were collected from a moving line during a five-hour period. During this time, 270 specimens were selected from approximately 2,200 (12.3%) pigs which weighed 180 to 220 pounds. It was estimated that the specimens obtained originated from at least 25 different sources.

A gross examination was made of the esophagogastric region, which was removed from the glandular portion, and cut into two parts. An attempt was made to obtain representative tissue on each of the two portions as determined by the gross examination. One part of the sample was tagged for later identification and fixed in 10% formalin which was changed periodically. The other part was placed in a labeled plastic bag, and temporarily put in a freezer box containing dry ice until these specimens could be stored at -20 C.

Gross Pathology. Studies of gross pathology were confined to the esophagogastric area. Denudation of the esophagogastric epithelium was measured as to the area of involvement and the depth of erosion. Hemorrhagic areas were considered evidence of subepithelial involvement. All observations were recorded as to type, location, and size.

Grades of Keratinization. The classification evaluation of amount of keratinization varied slightly from those employed by Griffing (1963). The following scale was used:

Grade K_0 - No keratinization visible grossly

Grade K_1 - Very slight keratinization

Grade K_2 - Slight keratinization

Grade K_3 - Moderate keratinization

Grade K_4 - Marked keratinization

Grades of keratinization were determined grossly and recorded for the 270 stomachs.

Mycology. Sabouraud's dextrose agar[1] was used to culture the tissues stored at -20 C. Chloramphenicol (0.05 mg./ml.) and cycloheximide[2] (0.5 mg./ml.) were used to inhibit bacterial and mold contamination. Pieces of tissue, 0.5 sq. cm. in size, were embedded in the agar. Six tubes of this medium were used for each specimen. Four tubes were incubated at 25 C., and two were incubated at 37 C. The tubes were examined daily for seven days, then twice a week thereafter. All culture tubes were observed for 30 days. All colonies appearing to be yeasts were Gram-stained. Colonies characterized by aerial hyphae were stained with lacto-phenol cotton blue. Upon microscopic examination, the stained preparations were identified as to genus when possible. The imperfect fungi were classified according to Barnett (1960). With the exception of the yeast-like organisms, no further identification was attempted. The yeast-like organisms characterized by being Gram-positive,

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1 Difco Laboratories, Detroit, Mich.

2 Actidone, Upjohn Co., Kalamazoo, Mich.

oval, budding, thin walled cells (2-5 microns) were purified as described by Laskowski et al. (1960). Confirmation of Candida spp. and identification of C. albicans was accomplished by the use of corn meal agar[1], Levine EMB agar[1], and sugar fermentation tests. Corn meal agar and Levine EMB agar were used as described by Griffing (1963). The procedure outlined by Laskowski et al. (1960) was used for the fermentation tests. By correlating the results of the various tests utilized, C. albicans could be positively identified.

Histopathology. Tissues collected for histopathological examination were fixed in 10% buffered formalin. Hematoxylin and eosin were used as the tissue stain, and Gridley's stain was employed to demonstrate fungi.

Controlled Experiment

History of Animals Used. The pigs selected for this experiment originated from three litters born and reared at the Kansas State University Veterinary Research Laboratory. Seven days separated the oldest and youngest litter. At approximately four weeks of age, the pigs were given access to a commercially prepared, pelleted ration[1] free of antibiotics and considered to have all known nutrients present. This ration was utilized throughout the experiment, and after weaning, was the only nutritional source.

At approximately six weeks of age, the pigs were vaccinated with modified-live hog cholera virus[2]. Anti-hog cholera serum[3] was given

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[1] Page's Complete Hog Feed, Manhattan Milling Co., Manhattan, Kans.

[2] Alocine, Haver-Lockhart Laboratories, Kansas City, Mo.

[3] Armour Pharmaceutical Co., Kankakee, Ill.

simultaneously. These animals were also vaccinated with erysipelas bacterin.[1]

Experimental Design. The pigs were divided into three groups. The selection was based upon weight, sex, and litter of origin. Group one was designated as the experimental group, group two as the surgical control group, and group three as the dietary control group.

Gastrotomies were performed on the pigs in group one. The esophagogastric epithelium was classified as to the amount of keratinization and denudation. The area was swabbed for culture purposes, mildly scarified, and innoculated with C. albicans. A 48-hour Sabouraud's dextrose agar culture of C. albicans suspended in 3 ml. of physiologic saline was used. Another swab was saturated with this suspension, and applied to the area.

At weekly intervals, for the first two months of the study, 40 ml. of physiologic saline with 50 million C. albicans cells per ml. were given by stomach tube. During the third and final month this dosage was given twice weekly to those pigs remaining on the experiment. The stomach tube was inserted into the esophagus to the thoracic inlet. Care was taken not to allow the stomach tube to enter the stomach.

Group two was subjected to the same surgical procedures and the esophagogastric epithelium was classified as to the degree of keratinization and denudation as in group one, except sterile physiologic saline was swabbed into the scarified area of the stomach. Forty ml. of physiologic saline was given by stomach tube at the same time group one was given C. albicans

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[1] Rhusigen, Pitman-Moore Co. Div., Allied Laboratories, Inc., Indianapolis, Ind.

and saline.

Group three was not subjected to surgery or to the use of the stomach tube. This group received the same diet as groups one and two.

Pentobarbital sodium[1] was the anesthetic used for the surgery in groups one and two.

Saline suspensions of C. albicans cells were counted by the Coulter electronic particle counter[2] and diluted to the desired concentration.

Nineteen pigs were to be used for this experiment; however, 2 pigs died during surgery. Of the remaining 17, there were 6 pigs in group one, 6 in group two, and 5 in group three.

Fecal Examination for Candida Species. Fecal specimens were taken three times before the experiment started and once a week during the experiment for each individual pig until each was euthanatized. These specimens were labeled and frozen at -20 C. until cultured. The time in frozen storage varied from one week to three months. Pagano Levine medium[3] was used as the selective medium for the determination of Candida species. Ten mg. of 2,3,5-triphenyl-tetrazolium chloride[4] and 50 mg. of neomycin were added to each liter of Pagano Levine medium. This medium was considered to be selective for the Candida species. The principle of this test as described by Pagano et al. (1957-58) is based upon the different capacities of Candida species to reduce T.T.C. and produce varying degrees of color.

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1 Halatol, Jensen-Salsbery Laboratories, Inc., Kansas City, Mo.
2 Coulter Electronics, Inc., Hialeah, Fla.
3 Difco Laboratories, Detroit, Mich.
4 T.T.C., Difco Laboratories, Detroit, Mich.

Pagano Levine medium was used as a quantitative test for Candida species as employed by Coles (1963). The fecal specimens stored at -20 C. were weighed aseptically into one-gram portions, and diluted in 9 ml. of sterile saline. One tenth and 1 ml. of thoroughly mixed suspension was placed into sterile petri dishes. Approximately 15 ml. of Pagano Levine medium was poured in each plate and mixed thoroughly. Duplicate plates were run on each dilution. After incubating for 48 hours at 37 C., smooth, round, glistening colonies with a cream to light pink color were counted. By this method, the relative number of Candida species in the feces could be determined. Periodic gram stains of colonies counted were made. The inhibitors in the medium were considered to be functioning properly when cells with the morphology characteristic of Candida species were obtained.

Blood Studies. Blood samples were taken three times before the onset of the experiment and once every two weeks thereafter. Samples were also taken immediately before necropsy. Disodium ethylenediaminetetraacetate[1] was used as an anticoagulant. The following tests were run: packed cell volume, hemoglobin, total WBC and differential WBC.

Necropsy Data. Pigs were randomly selected from each group for euthanasia at one month, two months, and three months after the beginning of the experiment (Table 1). A complete gross examination was made. Tissues were taken from all major organs, and fixed in 10% buffered formalin. Brain and kidney were taken aseptically and frozen at -20 C. for mycologic studies. Conant et al. (1954) reported isolations of Candida

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[1] EDTA, Cambridge Chemical Products, Inc., Dearborn, Mich.

species in these organs. The esophagogastric area of the stomach was observed and graded as to keratinization and amount of epithelial denudation. This tissue was collected for histopathologic and mycologic studies in the same manner described for collecting and processing the survey specimens.

TABLE 1—The Number of the Pigs Necropsied for Each Group During the Experiment

Group	1 month after surgery (pig No.)	2 months after surgery (pig No.)	3 months after surgery (pig No.)
1	33	13, 28, 26	18, 35
2	23	12, 17, 27	26, 31
3	25	32, 34	11, 21

Mycology. The same methods were employed for both culturing and identification as was described under survey specimens. The brain, kidney and esophagogastric epithelium were cultured.

Histopathology. Tissues from all major organs were saved for histopathologic studies. The esophagogastric areas of the stomach was treated in the same manner as described under histopathology of the survey specimens.

RESULTS

Survey Specimens

Gross Pathology. The 270 survey stomachs were classified as denuded or not denuded. There were 219 (81.1%) that showed no denudation, and 51

(18.9%) that showed denudation. Sub-epithelial petechiation was observed in 22 (8.1%) of the specimens and these were recorded as severe denudation. No ulcers were observed.

The amount of keratinization was categorized and classified. The keratin layer was considered by Smith and Jones (1961) to be the most superficial layer of stratified squamous epithelium. They stated that it may be considered abnormal if present in excessive amounts or in abnormal places. Seventy-seven (28.5%) of the survey stomachs showed no keratinization, while 193 (71.5%) showed varying amounts of keratinization. Some stomachs had excessive amounts of keratinized tissue over the entire esophageal area. Others had scattered wart-like projections at various places throughout with no or varying amounts of keratinization on the remaining tissue. In the latter case, only the wart-like projections were considered for keratinization classification. According to the grading used, there were 77 (28.5%) classified as K_0, 73 (27%) as K_1, 64 (23.7%) as K_2, 43 (15.9%) as K_3, and 13 (4.8%) as K_4.

The number of denuded stomachs for each grade of keratinization was determined (Table 2). For each grade increase in keratinization, denudation increased from 10.6% to 16.4%. The "row x column chi square test for proportionality" (Snedecor, 1956) was employed to test the increase in proportion (Table 3). At the 0.05 level, the grade K_1 was shown to have a significant increase in proportion of denudation than in grade K_0. The same statement could be made concerning the other groups having keratinization when comparing them to K_0. The results of this chi square test would indicate there is a relationship of keratinization to denudation, but the degree of keratinization is not necessarily a factor in this group.

TABLE 2—Number of Denuded Stomachs in Each Keratinization Groupings

Keratinization grade	No. in each group	Denudation present		Severely denuded	
		No.	%	No.	%
K_0	77	1	1.4	0	0
K_1	73	10	13.7	5	6.8
K_2	64	17	26.6	6	9.4
K_3	43	16	37.2	8	18.6
K_4	13	7	53.8	3	23.1
Totals	270	51		22	
Total % denudation			18.9		8.1

TABLE 3—Results of Row x Column Chi^2 Test of Proportionality.
= .05 Reject when chi^2 3.84

H_0:	Chi^2 values	Conclusion	H_0:	Chi^2 values	Conclusion
$K_0 = K_1$	8.30	reject	$K_1 = K_2$	3.76	accept
$K_0 = K_2$	20.85	reject	$K_2 = K_3$	1.25	accept
$K_0 = K_3$	29.90	reject	$K_3 = K_4$	0.60	accept
$K_0 = K_4$	44.60	reject			

Mycology. Of the 270 stomachs collected, 50 stomachs were selected randomly for mycological studies. The amount of keratinization and denudation is representative of the 270 observed on gross examination.

There were a variety of organisms isolated as shown in Table 4. With the exception of Candida and Trichosporon species, the fungal isolations were identified upon initial isolation by colony and microscopic morphologic characteristics. Trichosporon was identified on the special media used to identify the Candida species.

TABLE 4—A Listing of the Various Organisms Cultured During Mycological Studies from Esophagogastric Area

Genera	No. isolated	Genera	No. isolated
Streptomyces species	21	Trichosporon species	3
Geotrichun species	9	Aspergillus species	2
Candida species*	8	Mycelia sterila	3
Penicillium species	3		

* Two isolates were identified as C. albicans.

Candida species and C. albicans were primarily considered in this survey. There were 8 (16%) Candida species isolated. Two (4%) were later identified as C. albicans.

Of the 8 Candida isolates, an inconsistency existed between the isolation of the organism and the degree of keratinization present (Table 5). In comparing the Candida species cultured to the amounts of keratinization present, 3 were isolated from 17 specimens classified as K_0, 3 from 11 classified as K_1, and 2 of 6 from K_3. No isolates were made from the K_2

on K_4 stomachs (Table 5). Denudation was observed in one stomach from which a Candida isolate was made. It was graded as K_3.

Two isolates were C. albicans. The keratinization grades were K_0 and K_1. No denudation was noted in these two specimens.

TABLE 5—Summary of the Relationship of Keratinization and Epithelial Denudation to the Incidence of Candida Species

Keratinization			Denuded epithelium		Incidence of Candida species		
grade	No.	%	No.	%	Mycologic	Histopathologic (Gridley's)	Total
K_0	17	34	1	2	3*	1	3**
K_1	11	22	0	0	3*	1	4
K_2	13	26	2	4	0	1	1
K_3	6	12	3	6	2	0	2
K_4	3	6	2	4	0	0	0
Total	50	-	8	16	8	3	10

* Includes one isolate of C. albicans; no denudation observed.
** One positive for both methods employed.

Histopathology. Microscopic examination was made of the same 50 specimens utilized for mycologic examinations. Sections of the stomachs were stained with haematoxylin and eosin, and Gridley's stain.

The stratified squamous epithelium of the esophageal region was often characterized by accumulation of keratin on the surface. Nuclear retention was observed in the keratin layer. A layer of large, clear cells was observed in the epithelium of many specimens which did not stain with the methods employed. No significance was attached to these cells.

Forty-one of the 50 tissues examined microscopically appeared normal. The rete pegs were normal in length, and evidence of inflammatory cells or vascular changes were not observed.

Denuded epithelium was noted on six stomachs in which the lamina propria was exposed. A mild infiltrative process characterized by an increase in eosinophils, neutrophils, and lymphocytes was observed in four of the six specimens. In these six specimens, basal cell proliferation was evidenced by the downward extension of the rete pegs.

Three of the tissues observed had inflammatory changes in the propria; however, no pathologic changes were seen in the stratified squamous epithelium.

Pseudohyphae and budding yeast cells were observed in three of the 50 sections stained by Gridley's technic and were tentatively identified as Candida species. Ajello et al. (not dated) considers the finding of pseudohyphae and budding yeast cells simultaneously present in tissue to be diagnostic of Candida species.

Controlled Experiment

Fecal Examination for Candida Species. Fecal specimens were taken one week previous to the beginning of the experiment and at regular intervals during the experiment. During the first two months of the experiment, the fecal specimens were collected seven days after the animals in group one were given C. albicans, and during the third month, the specimens were collected four days after the animals in group one were given C. albicans.

No apparent increase of Candida species was observed in the C. albicans group over the control groups not receiving C. albicans during the first 60 days of the experiment. During the final 30 days of the experiment, all specimens from group one were positive for Candida species; however, in group three, 70% of the samples taken were positive (Table 6).

Blood Studies. Packed cell volume, hemoglobin, total WBC and WBC differential were run for each pig on three blood samples before the experiment and every two weeks during the experiment. All findings were considered to be in the normal range for swine. Data compiled for individuals and as group means showed no significant difference between the groups.

Necropsy Data. Pigs were necropsied at various times during the experiment (Table 1). There were no gross lesions or disease processes observed at necropsy which were considered to have affected the outcome of the experiment.

A careful examination of the esophagogastric area revealed pathologic changes which were not confined to any one group. All degrees of keratinization were observed, irrespective of the groups from which they were derived. Denudation was present in 5 of the 6 pigs in group one, 5 of the 6 pigs in group two, and 3 of the 5 pigs in group three (Table 7). Subepithelial hemorrhage was observed in 1 pig from group one, 2 from group two, and 1 from group three.

An ulcer was observed from pig No. 36 in group one. The lesion was 1 cm. x 2 cm. in size. The edges were slightly elevated, and the base was brown and rough. A similar lesion was observed from pig No. 17 in group

TABLE 7—Gross Observations of the Esophageal Area of the Stomach at the Time of Surgery and Necropsy

Pig No.	Keratinization		Denudation		Ulceration
	Surgery	Necropsy	Surgery	Necropsy	(Necropsy)
			Group 1		
33	K_2	K_0	0	+	0
13	K_2	K_1	+	+	0
28	K_2	K_4	0	+	0
36	K_3	K_3	0	+	+
18	K_3	K_3	+	+	0
33	K_2	K_3	0	0	0
			Group 2		
23	K_2	K_4	0	+	0
12	K_2	K_1	0	+	0
17	K_1	K_2	0	+	+
27	K_2	K_3	0	+	0
26	K_3	K_1	+	+	0*
31	K_1	K_3	0	0	0
			Group 3		
25	-	K_4	-	+	0
32	-	K_4	-	0	0
34	-	K_4	-	0	0
11	-	K_2	-	+	0
21	-	K_1	-	+	0

* Small, white, glistening area, considered to be a healed ulcer.

two which was 1 cm. in diameter. Pig No. 26 in group two exhibited a small, white, glistening area 0.5 cm. in diameter which was considered to be a healed ulcer (Table 7).

Mycology. At the time of surgery, the esophagogastric epithelium was swabbed for culture purposes on those pigs subjected to surgery. Identification of Candida species and C. albicans were carried out. In group one, two Candida species plus one C. albicans were found. In group two, one Candida species plus three C. albicans were identified (Table 8).

At necropsy, mycologic examination of the esophagogastric area revealed the presence of six C. albicans: four were from group one, one from group two, and one from group three. Five other Candida species, not C. albicans, were isolated (Table 8). These Candida species were found in all 6 animals of group one, 4 of 6 in group two, and in 4 of 5 from group three.

Kidneys and brains of all animals were cultured, and no isolations were made.

Geotrichum species was isolated from eight swabs taken at the time of surgery. At necropsy, three isolates of Geotrichum species were made.

Histopathology. Tissues collected for histopathologic examination were stained with Gridley's stain and examined. Four observations of pseudohyphae and budding yeast cells were observed. As previously stated, these were tentatively considered as Candida species. These cells were found in the outermost portion of the keratin layer. Only one of these was positive by the culture method (Table 8). Sections stained with haematoxylin and eosin from each stomach were examined. Uniform findings

TABLE 8—Summary of the Mycologic Studies at the Time of Surgery and Necropsy (Gridley's Stain Included)

Pig No.	Time of Surgery		Time of Necropsy			
	Mycologic		Mycologic		Gridley's*	Total
	C. albicans	Candida species	C. albicans	Candida species	Candida species	Candida species
			Group 1			
33	0	0	0	+	0	+
13	0	+	+	+	0	+
28	0	0	0	+	0	+
36	0	0	+	+	0	+
18	+	+	+	+	0	+
35	0	+	+	+	0	+
			Group 2			
23	0	+	0	0	+	+
12	0	0	0	0	0	0
17	+	+	0	0	0	0
27	+	+	+	+	+	+
26	0	0	0	+	0	+
31	+	+	0	0	+	+
			Group 3			
25	-	-	0	+	0	+
32	-	-	0	+	0	+
34	-	-	+	+	0	+
11	-	-	0	0	+	+
21	-	-	0	0	0	0
Total	4	7	6	11	4	14

* Budding yeast and pseudohyphae observed.

were observed throughout the animals irrespective of groups. Denudation, as described previously, was found in all but three tissues examined. Infiltration of neutrophils, eosinophils, and lymphocytes was mild to moderate in all specimens with exception of two. The other histologic alteration observed was extension of rete pegs into the lamina propria.

DISCUSSION

Studies were conducted which showed an epidemiologic relationship of esophagogastric epithelial denudation and ulceration to the incidence of Candida species, especially C. albicans. In the survey group, there were no esophagogastric ulcers demonstrated and 18.9% denudation observed. The mycologic incidence was 16% Candida species and 4% C. albicans. In the experimental pigs, the findings were: 11.8% ulcers, 76.5% denudation, 82.4% Candida species, and 35.3% C. albicans. When considering the etiology of esophagogastric ulcers, the presence of Candida species and C. albicans should be considered, at least as a contributing factor.

In a similar survey, Griffing (1963), found no esophagogastric ulcers, 8% denudation, 27% Candida species, and no C. albicans. The Candida species isolated was largely C. zeylanoides, which he considered to have no pathologic significance. Disregarding C. zeylanoides, the incidences of Candida species as related to denudation and ulceration, are similar in the two surveys. The same relationship exists when comparing the incidence of C. albicans.

Baker and Cadman (1963), Curtin et al. (1963) and Griffing (1963) were able to isolate C. albicans routinely from esophagogastric ulcers. The

findings in this experiment further demonstrate the relationship of candidiasis to esophagogastric ulcers.

When comparing the survey animals to the experimental pigs, no relationship as to diet was determined. The survey animals originated from a number of sources, and the physical and chemical characteristics of the feed undoubtedly varied from animal to animal, depending upon the source. There were no antibiotics or sugar added to the feed of the experimental pigs.

Seasonal occurrence, as mentioned by Kowalczyk (1960) and Curtin et al. (1963) was not considered in the evaluation because all stomach specimens were obtained during the same season of the year.

Berg (1960) and Osborne et al. (1960) considered various environmental factors may be of importance to esophagogastric ulcers. It was felt that these factors should not be overlooked. The experimental groups were subjected to surgery, the collection of fecal specimens from the rectum, the collection of blood, and the withholding of feed. Pigs having a high incidence of ulcers have been derived from swine testing stations (Griffing, 1963; Perry et al., 1963) or experimental animals which have been handled or treated quite frequently (Kowalczyk, 1960; Buntain, 1961). Such factors may have contributed to the high incidence of denudation and ulceration in the experimental groups.

In these studies, keratinization was considered as a factor in the genesis of esophagogastric ulcers. Denudation, which was considered as possible predisposing factors contributing to subsequent ulceration, was shown statistically to be related to keratinization. Kowalczyk (1960) observed erosion of the epithelium by gastroscopic methods.

It was concluded that Candida species, keratinization and environmental factors contributed to the pathologic findings of esophagogastric area in the pigs studied. Other factors which have been stated previously as possible causes or contributing factors had no apparent relationship to the finding in this experiment.

In future studies, the use of Candida-free pigs must be considered. Gastroscopic examination of the esophagogastric epithelium during experimentation may demonstrate valuable information necessary to determine the pathogenesis and etiology of esophagogastric ulcers.

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THE EFFECTS OF *CANDIDA ALBICANS* ON THE ESOPHAGOGASTRIC AREA OF THE PORCINE STOMACH

by

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In a randomized sample of 270 swine stomachs taken during one day's slaughter at a Kansas City, Kansas abattoir, excessive amounts of keratinization of the esophagogastric epithelium was observed in a small number of animals (2.9%).

Denudation was noted in 51 (18.9%) of the tissues observed. When applying the "row x column chi square test for proportionality", a relationship of keratinization to denudation existed, but the degree of keratinization was not proven to be a factor. Denudation was considered as possible predisposing factors contributing to subsequent ulceration.

Fifty of the 270 tissues studied by mycologic and histopathologic methods revealed a 16% incidence of Candida species and a 4% incidence of C. albicans. Forty-one stomachs were considered to be normal when examined histopathologically. Denuded epithelium was noted in six tissues, and a mild infiltrative process of the properia was observed in seven tissues.

In an experiment designed to study the effects of C. albicans on the esophagogastric epithelium, 8 of the 11 control animals (groups two and three) were infected from unknown sources throughout the experiment with Candida species including C. albicans. The incidence of Candida species in the controlled experiment was 14 to 17 (82.4%), while six of 17 (35.3%) tissues were positive for C. albicans. Gross and histopathologic findings revealed denudation and/or ulceration in all groups. It should be pointed out that six of 17 (35.3%) tissues were positive for C. albicans in the experimental groups, while only 2 of 50 (4%) were positive for C. albicans in the survey group. The incidence of Candida species

in the controlled experiment was 14 of 17 (82.4%) while the survey group showed 8 in 50 (16%). This evidence indicates a possible relationship of Candida species to denudation and ulceration of the esophagogastric epithelium. However, the surgery at the onset of the experiment, collecting fecal specimens from the rectum, collecting blood for study, and withholding feed may be factors effecting the findings in the esophagogastric area. Such factors should be considered.