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EXPERIMENTAL REPRODUCTION OF EDEMA DISEASE OF SWINE[1]

IN-CHANG PAN[2], CHUN-TSUEN WANG[3], YAU-CHIN YEH[4], IN-JEN PAN[5] AND HSIEN-CHUAN CHEN[6]

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Many authors (1-20, 25) reported that hemolytic *E. coli* was frequently associated with edema disease of swine (ED); however, reproduction of the disease, using hemolytic *E. coli* strains isolated from natural cases, was not always successful. Some authors (1, 6, 8, 20-24) claimed that the disease was constantly reproducible in susceptible pigs by intravenous inoculation of extracts of intestinal contents collected from natural cases. They suggested that the disease was caused by toxemia. However, histopathological identification of naturally affected animals and experimentally reproduced ones has not been throughly carried out in these studies, thus it is dubious whether the disease has actually been successfully reproduced.

In a previous paper (26) Pan pointed out that the disease was characterized by the damage of small vascular walls, which caused circulatory and permeability disturbances followed by edema. The characteristic features of fibrinoid necrosis in the small vascular walls, fibrin thrombus formation and atony of capillaries, and extensive eosinophilia, closely resemble the pathological changes in tissue allergy. The rapid fatal course of the affected animals, after the onset of symptoms, would seem to support this observation.

Therefore, a study of the etiology and pathogenesis of ED was undertaken. The present report is confined mainly to the experimental reproduction of ED in pigs.

MATERIALS AND METHODS

Experimental reproduction of ED. Seventy-five healthy pigs, purchased from local farms, weighing from 26 to 40 lbs were used. Only littermates were used in any single experiment whenever possible. 6 experiments were made. Details of inoculation procedures will be described separately in each experiment.

Histopathology of experimental cases. For identification and confirmation of the lesions of experimentally induced cases, histopathological studies were made. Specimens were collected and fixed in formal-alcohol which consisted of equal volumes of 20% formalin and 95% alcohol. For routine examinations, paraffin sections were cut 6μ thick and stained with hematoxylin and eosin.

Isolation and identification of hemolytic E. coli. Ten per cent goat or sheep blood agar plates were used for the screening purpose. All viscera, including intestinal contents, of both natural and experimental cases were examined for hemolytic *E. coli.* Samples were cultured on blood agar plates and were incubated at 37 C for 18 hours. The colonies which showed marked β type hemolysis were isolated and transferred to 10% blood agar slants. McConkey, E. M. B., and S. S. agar plates were used for preliminary identification of *E. coli* and for screening possible Salmonella contaminants. Identification of the isolates was based on morphological examination, the growth condition on Kligler and Simon's citrate agar slants, indol formation, methyl red test, Voges-Proskauer reaction, *etc.* as generally required.

RESULTS

Experimental Reproduction of ED

Experiment 1. This experiment served as a pilot test for detecting a suitable dosage of inoculum to reproduce ED. 14 pigs were used in this experiment. Intestinal contents collected from naturally affected animals were diluted with normal saline in various proportions. The diluted materials were extracted in refrigerator overnight, and the supernatants were used as inocula after centrifugation at 4,000 rpm for 30 minutes. The results are shown in Table I.

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2 Lecturer, Department of Veterinary Medicine, College of Agriculture, National Taiwan University, Taipei, Taiwan.

3, 4 Junior technicians.

5, 6 Research assistants.

TABLE I

Detection of a suitable dosage of inoculum to reproduce ED

Recipient pig	Donor pig	Inoculum		Inoc. route	Toxicity			ED
		Volume ml	Dilution Saline/Material		Grade	Surv.	Death	
A	6008	15	9/1 (Seitz filt. small)	iv	++	+		−
	6007	20	1/1 (small)	iv	−	+		−
B	6008	20	9/1 (Seitz filt. small)	iv	++	+		−
	6007	20	1/1 (small)	iv	−	+		−
C	6007	18.5	1/1 (small)	iv	+	+		−
	6007	27	1/1 (small)	iv	+	+		−
D	6007	20	1/1 (small)	iv	−	+		−
	6007	20	1/1 (small)	iv	−	+		−
E	6013	25	1/1 (small)	iv	−	+		−
F	6013	30	1/1 (small)	iv	−	+		−
6014	6013	30	1/1 (large)	iv	++	+		−
6018	6016+6017	7.5	1/1 (small)	iv	+++		+(2 hrs)	−
G	6016	15	1/1 (large)	iv	+++	+		−
6019	6016	15	1/1 (large)	iv	+++	+		−
H	6020	20	1/1 (small)	ip	−	+		−
6021	6020	15	1/2 (large)	iv	++		+(36 hrs)	+
6022	6013	10	whole blood	per os	−	+		−
	6020	13	1/2 (large)	iv	++		+(30 hrs)	+
6023	6017	100	5 ml/plate	ii	−	+		−
	6020	11.5	1/2 (large)	iv	++		+(60 hrs)	+

Small: Small intestinal content.
Large: Large intestinal content.
ii: Intra-small intestinal inoculation conducted under surgical conditions.
Plate: Blood agar plate culture of hemolytic *E. coli*.
Receipment pigs, A-D, 6022 & 6023, each received 2 inoculations. The 1st inoculation was followed by the 2nd at an interval of 14 days.

As shown in the table, 3 cases of edema disease were successfully reproduced by inoculating 11.5 ml or more supernatant of 66% diluted contents of the large intestines of diseased pigs. Only signs of intoxication were induced, but not the disease itself, when higher dilutions were inoculated. Under the condition of the experiment, no ED occurred when larger volume of 50% dilution of the contents of the small intestines was used as inoculum.

Experiment 2. In the previous experiment, we failed to detect the proper dosage of the content of small intestines which was capable of reproducing ED. Experiment 2 was accordingly performed with 9 pigs to solve this point. The results obtained are shown in Table II.

From the Table, it is apparent that this disease was reproducd in healthy pigs by inoculating 7.5 ml or more of 75% diluted contents of the small intestines of diseased pigs, under the experimental conditions specified. The animals which were inoculated with a small dose of the extract of large intestinal content died earlier than those inoculated with a larger dose of extract prepared from the small intestinal content. It appears that some substances other than ED producing factor might be the cause of this difference.

2 pigs were inoculated with the content of small intestines of a normal pig which was a hemolytic *E. coli* bearer at the time of the experiment. The purpose was to see whether the intestinal content of a pig, which harboured hemolytic *E. coli*, contained ED causing factor. No untoward reactions were observed.

Antihistaminics (Vena and Allermin*) were given to pig 6034. A total of 150 mg of Vena

*Vena: Diphenhydramine hydrochloride (Tanabe Pharm. Co. Ltd., Japan); Allermine: Chlorprophenpyridamine maleate (China Chemical & Pharm. Co. Ltd., Taiwan).

TABLE II

Reproduction of ED by intravenous inoculation with supernatant of intestinal contents of diseased pigs

Recipient pig	Donor pig	Inoculum		Edema disease	
		Volume ml	Dilution Saline/Material	Incidence	Time confirmed
6035	Mixture from 6025, 6026, and 6027	16.5	1/3 (small)	+	15 days
6030		11	1/3 (small)	+	66 hrs
6028		12	1/2 (large)	+	12 hrs
6029		10	1/2 (large)	+	20 hrs
6034		12	1/3 (small)	+	5 days
6031		11.5	1/3 (small)	+	15 hrs
6032		7.5	1/3 (small)	+	43 hrs
I	6095*	20	1/3 (small)	−	
J		20	1/3 (small)	−	

* A normal pig which beared hemolytic *E. coli* when the intestinal content was collected.

and 30 mg of Allermin were injected intravenously, within 4 successive days. This animal survived for 5 days, while the other 2 non-treated animals which received smaller inoculum died within 3 days after inoculation. Pig 6034 survived longer probably by virtue of antihistaminics, since the animal died the next day after interruption of drug administration.

Experiment 3. This experiment was conducted to see whether ED could be reproduced in pigs by inoculating frozen-thawed hemolytic *E. coli,* as claimed by some workers (1, 3, 5, 6, 17, 29). 13 pigs were used in this experiment. 8 strains of organisms isolated from natural cases of this disease were used. 1-day old blood agar cultures were suspended in normal saline to a turbidity corresponding to No. 10 of MacFarland's nephrometer. Organisms were frozen-thawed 20 times in a dry-ice-alcohol mixture, and then centrifuged at 4,000 rpm for 30 minutes, and the supernatant was used as the inoculum. An aliquot of untreated bacterial suspension was used for comparison. Table III shows that among 9 animals inoculated with endotoxin, 5 animals

TABLE III

Inability of hemolytic E. coli endotoxin to produce edema disease

Pig no.	Organisms from	Inoculation			Toxicity			ED
		Dosage ml	Material	Route	Grade	Surv.	Sacrif.	
L	6001	10	endotoxin*	iv	−	+		−
E3		10	endotoxin	iv	−	+		−
M	6013	13	endoxtoin	iv	+	+		−
6015		20	endotoxin	iv	+++		28 hrs	−
E8		200	untreated**	per os	−	+		−
E9		200	untreated	per os	−	+		−
N	6017	100	untreated	per os	−	+		−
E10		100	untreated	ii***	−	+		−
E4	6008	10	endotoxin	iv	−	+		−
E5		7.5	endotoxin	iv	−	+		−
6036	6020, 6025	18	endotoxin	iv	+++		5 days	−
O	6026 & 6027	16	endotoxin	iv	+++	+		−
P	mixture	12	endotoxin	iv	+++	+		−

* The bacterial suspension was adjusted to a turbidity corresponding to No. 10 of MacFarland's nephrometer, and then frozen-thawed for 20 times.
** Untreated bacterial suspension with the same turbidity as above.
*** ii: Intra-intestinal inoculation.

showed marked excitement, followed by marked depression, but most of them recovered within 3 days after inoculation. 2 of these animals (6015 and 6036) which received the largest inoculum were killed in the depressed condition and examined. No evidence of ED was observed other than edematous condition of the connective tissue around the gall bladder. Histopathologically, case 6036 showed deposition of fibrinoid material in the medium-sized arteries: cavanous sinuses of the hypophyseal fossa in the sphenoid bone, one interlobular artery of the kidney, and one artery in the adrenal body (Fig. 1). In both cases, loosened and edematous arteries were found everywhere. The significance of these lesions will be discussed later. The other animals which received untreated bacterial suspension by oral or intestinal routes did not show any unfavorable signs after inoculation.

Experiment 4. The purpose of this experiment was to see if the ED causing factor could be adsorbed by aluminum hydroxide-gel (33 mg/ml). 18 pigs were used in this experiment. The supernatant of the extract of the small intestinal contents was prepared in the same manner as described in Experiment 2, except that the concentration was doubled. An equal amount of aluminum hydroxide-gel (pH 7.0) was added to the supernatant of the extract, and the mixture was allowed to stand for 1 hour in the refrigerator. After centrifugation at 3,000 rpm for 30 minutes, the supernatant was used for inoculation. 6 animals received adsorbed inocula, while 4 animals (cases 6043, 6045, 6047 and Q) received non-adsorbed materials for control purposes. The results are shown in Table IV.

TABLE IV

Adsorption of ED-producing factor in supernatant of intestinal contents by aluminum hydroxide-gel

Recip. pig	Donor pig	Inoculation, iv		Edema disease			
		Volume ml	Dilution, Saline/Material	Symptoms	Incidence	Time confirmed	Fate
6048	Mixture	29.5	1/3(small, adsorbed)	+	+	42 hrs†	D.
6046	from	20	1/3(small, adsorbed)	+	−	18 hrs	D.
6051	6037,	25	1/3(small, adsorbed)	−	−	9 hrs	K.
6052	6039,	25	1/3(small, adsorbed)	+	−	24 hrs	K.
6045	6040	17	1/3(small, non-adsorbed)	+	+	16 hrs	D.
6047	and	17	1/3(small, non-adsorbed)	+	+	12 hrs	D.
6043*	6041	19	1/3(small, non-adsorbed)	+	+	48 hrs	K.
Q		10	1/3(small, non-adsorbed)	−	−	5 days	R.
6267		6	1/3(small, non-adsorbed)	+	+	7 days	K.
6255		10	1/3(small, non-adsorbed)	−	−	15 hrs	D.
6258		15	1/3(small, non-adsorbed)	−	−	15 hrs	D.
6259	Mixture	20	1/3(small, non-adsorbed)	−	−	15 hrs	D.
6270	from	8.85**	1/3(small, non-adsorbed)	+	+	58 hrs	D.
6263	30 cases	12	1/3(small, adsorbed)	−	−	1 hr	D.
F1		10	1/3(small, adsorbed)	−	−	12 hrs	R.
F2		20	1/3(small, adsorbed)††	−	−	20 hrs	R.
F3		25	1/3(small, adsorbed)††	−	−	1 hr	R.
F4		12	1/3(small, adsorbed)††	−	−	1 hr	R.

D. Died naturally.
K. Killed for examination.
R. Recovered from intoxication symptoms.
* Had received 30 ml supernatant of *E. coli* culture, which was cultured in 0.2 mol/ml histidine solution, 9 days previously without any unfavorable signs.
** Mixed with 13.3 mg of Allermin.
† Time elapsed after inoculation.
†† Sediment of the aluminum hydroxide treated extract was adjusted to pH 8.0 before further processing.

The table shows that the supernatant of aluminum hydroxide-gel-adsorbed contents of the small intestines was not potent in producing ED. Only one out of 6 animals tested (case 6048) was confirmed to acquire ED, and the lesions observed were not as pronounced as those produced by non-adsorbed materials. The data suggest that the ED causing factor can be adsorbed by aluminum hydroxide-gel. If this is true, then the ED observed in pig 6048 might have been caused by materials which escaped adsorption somehow.

Three animals were inoculated with materials prepared in the following manner. The aluminum hydroxide-treated extract was centrifuged and the supernatant discarded, and the sediment was brought to pH 8.0 with phosphate buffer (M/15), stirred, and allowed to stand at 4 C for 10 minutes. After centrifugation at 3,000 rpm for 30 minutes, the supernatant was used as inoculum. Table IV indicates that elution of the ED causing factor failed to occur when pH was adjusted to 8.0.

Experiment 5. This experiment was made to find out the cause of sudden death of animals after they received either adsorbed or non-adsorbed inoculum. Many animals died within a short time after inoculation, even though the dose inoculated was insufficient to produce ED. 2 experiments were conducted, and the results are summarized in Table V. The inoculum was prepared as described in Experiment 4.

TABLE V

Nature of a toxic factor in the supernatant of bowel extract adsorbed with aluminum hydroxide-gel

Recip. pig	Source of extract	Inoculation, iv		Result
		Volume ml	Dilution Saline/Material	
T	Mixture	20	1/3 (small, adsorbed)	Marked intoxication. Reching, constricted pupils, bradycardia in 5 min. followed by tachycardia, died within 10 min. after injection.
U	from	15	ibid	
V	6073,	9	ibid	
W	6074,	5	ibid	
X	6083	3	ibid	
Y	and	4	ibid (with 4 ml drug)*	Slight anorexia. Recovered in 2 days.
Z	6089	18	ibid (with 11 ml drug)*	Staggering gait, and vomitting. Recovered in 2 days.
6064	Mixture	20	1/4 (small, adsorbed)	Marked intoxication, died in 20 min.
6065	from	13	ibid	Edema of stomachs was marked.
6066	6060,	11	ibid	
6070		17	1/3 (small, adsorbed)	Marked intoxication, died 30 min. after injection.
S	6061	15	ibid	Marked intoxication, recovered 3 days after injection.
6075	and	20	ibid, dialysed***	Marked intoxication, jumping motion. Died in 1 hr.
F5	6062	20	ibid***	Marked intoxication, recovered 6 days after injection.
6076		5	40 mg/ml	Marked intoxication. Constricted pupils, bradycardia followed by tachycardia with extreme agony. Died within 5 min. after injection.
6077		2.5	40 mg/ml	
6078	Histamine	1	40 mg/ml	
F6	phos-	4	5 mg/ml	Moderately depressed. Anorexia followed but recovered 3 days after injection.
F7	phate**	2	5 mg/ml	
F8		1	5 mg/ml	
F9		0.5	5 mg/ml	Negative signs.

* Each ml of drug contained 20 mg Allermin (Chlorprophen-pyridamine Maleate) and 200 mg Caffeine Sodium Benzoate, prepared by China Chemical and Pharmaceutical Co. Ltd., Taiwan.

** A product of Kanto Pharmaceutical Co., Japan.

*** After adsorption of the original bowel extract by an equall volume of aluminum hydroxide-gel (33mg/ml), a supernatant was obtained by centrifugation at 3,000 rpm for 30 minutes. The supernatant was then dialysed in running water for 6 hours.

In the first experiment, the lethal dose of aluminum hydroxide-gel-adsorbed inoculum was determined in 6 animals, and it was found that 3 ml of inoculum given intravenously proved to be lethal. 2 animals received a mixture of adsorbed material and the antihistaminic drug (Allermin). Table V shows clearly that the toxic action of adsorbed inocula was inhibited almost completely by the simultaneous administration of Allermin: animal Z which had received an inoculum 6 times stronger than the lethal dose was saved by the administration of Allermin. It leaves no doubt that the toxicity of the inoculum was related to histamine.

Here we faced the question of whether histamine existed as such in the adsorbed inoculum. The adsorbed material was first dialysed against running water overnight; after centrifugation at 3,000 rpm for 30 minutes, the supernatant was used as inoculum. The result shows that the toxic factor could not be removed by dialysis, *i.e.* the toxic factor was a large molecular substance, and that the toxic action was not due to pre-existing histamine in the inoculum. Millon's reaction was positive for the dialysed inoculum.

Three pigs (6064, 6065, and 6066) received 11 to 20 ml of non-dialysed inoculum each. The animals died within 20 minutes after inoculation. Marked submucosal edema of the stomach appeared in all animals. Histopathologically, however, no vascular lesions characteristic in ED were demonstrable.

The main purpose of the second experiment was to determine the degree of susceptibility of our pigs to histamine, and to see if there exists lesional similarity between animals inoculated with histmaine and with gel-adsorbed inoculum. It was found that pigs were highly sensitive to histamine inoculation. A small histamine dose of 2.4 mg/kg body weight sufficed to kill an animal within 5 minutes after injection. Clinical signs were more or less similar to those of animals receiving intestinal content intravenously. Histopathologically, the same lesions appeared in the arteries of animals in both groups, with the majority of lesions in medium-sized arterial walls. Smooth muscle bundles were separated from each other by clear spaces, presumably due to edematous changes in the ground substance (Fig. 2 and 3). Edema was always demonstrable in the lymph sinuses of the lymph nodes and in the perivascular space of the brain. Marked atony and hyperemia of capillaries, and occasional thrombus formation, were observed (Fig. 4).

Experiment 6. It is obvious from the experiments mentioned above that many animals died with symptoms simulating histamine-shock shortly after inoculation, and that early death could be prevented by the simultaneous use of antihistaminic drugs. Here a question arises as to whether hemolytic *E. coli* in intestines possesses decarboxylase and converts histidine, a predominant amino acid constituent of soy-bean-meal which is the main feed for pigs on Taiwan, to histamine which then acts as a preformed toxin. The following experiment was designed to solve this point.

10 strains of young agar cultures of hemolytic *E. coli* were suspended in buffered saline (pH 7.0), and then washed 3 times with chilled buffered saline, by centrifugation, to get a final suspension in saline (0.2 mol) solution of histidine with a density of 200 billion cells/ml. Histidine was prepared at the concentration of 0.2 mol (31 mg/ml in distilled water). Bacterial suspension without histidine, and plain distilled water were set up for control purposes. The experimerital suspensions and controls were kept in the Warburg manometer (37 C) for measuring CO_2 formation. Readings were made at intervals of 40 minutes to 2 hours. During the observation period of 16 hours, no meaningful change in gas pressure was observed. One pig (6043) was then inoculated with 30 ml of supernatant of a bacterial suspension kept in histidine-saline at 37 C for 24 hours. Another pig (O) which received 13 ml of histidine-saline alone served as the control. Both animals failed to show any abnormal symptoms during the observation period of 10 days. It appears from this experiment that the strains of hemolytic *E. coli* examined did not produce decarboxylase. Therefore, it is reasonably certain that the toxic factor in our inocula could hardly be preformed histamine in the gut produced by hemolytic *E. coli*.

Symptomatology and Pathology of Experimentally Reproduced ED

Seventeen cases of ED were reproduced successfully in this study. Since the findings on necropsy and histopathology on experimentally reproduced ED will be described in detail in a separate paper, only brief points are given here.

Symptomatology. Animals usually developed marked signs of intoxication, *e.g.* vomiting, a staggering gait, falling upon the ground with groans, and convulsions right after the inoculation. The susceptibility of individual pigs to the same dose of inoculum seemed to vary, and we stopped inoculation when an animal stretched its nasal wings and its skin started turning red-prodromes which were generally followed by extreme

excitement. Anesthesia was not used throughout our experiments. In severe cases, animals usually died 10 minutes to 1 hour after inoculation, depending on the tolerance of the animals and the dosage of inoculum given. The toxicity of the inoculum could be alleviated when antihistaminics were used simultaneously. Those animals which survived this critical period generally developed typical symptoms of edema disease of swine: a staggering gait, swollen eye lids, and sudden falling upon the floor with severe galloping-type motions of the lower limbs within 20 hours after inoculation. Seldom did the animals die during this primary attack; they usually recovered some ten minutes later. In these cases, the nervous signs reappeared several times a day. Most of the animals died from ED within 48 hours after inoculation, with the exception of cases 6034 and 6035 which were sacrificed 5 and 15 days after inoculation respectively. Case 6034 was treated with antihistaminics and antibiotics for 5 days; the symptoms always improved immediately after administration of antihistaminics, only to reappear 2 or 3 hours later. It was sacrificed during the moribund stage after discontinuance of antihistaminics administration.

Case 6035 was sacrificed 15 days after inoculation. The animal showed marked depression and vomiting right after inoculation. Anorexia followed. She lay on the straw mat continuously during the observation period. A slight staggering gait, swollen eye lids, and a moderately swollen head and face appeared 6 days later. Edema decreased a little on the day when she was sacrificed.

General gross findings. The lesions which appeared in reproduced cases of ED were identical with those of natural cases. Edematous change of the stomach appeared in 83% of the animals, which occurred mainly in the submucosa of the cardiac gland portion (Fig. 5). Edema was also found in the mesentery of the coiled colon of 60% of the animals. Yellowish, fine fibrin threads in the peritoneal cavity and pericardiac sac were found in all animals. The lung was always edematous and congestive.

Usually, no significant gross lesions were found in the intestines; however, the terminal ileum and large intestine of case 6034 were markedly hemorrhagic and necrotic.

All lymph nodes showed moderately swollen and hemorrhagic periphery.

No particular changes were noted in other viscera.

General histopathological findings. The lesions were essentially identical with those of natural cases. The distribution of vascular lesions in various organs is shown in Table VI. The characteristic changes were fibrinoid necrosis, edematous swelling and fibrin thrombi, seen in

TABLE VI

Distribution of characteristic lesions in experimentally produced ED

Pig no.	Edema		Vascular lesions (fibrinoid necrosis, edematous swelling, fibrin thrombi etc.)						
	Stomach	Mesentery	Lymph nodes	Heart	Stomach	Intestine	Liver	Kidney	Larynx
6019	−	−	+	−	−	−	+	+	−
6021	+	+	+	−	−	+	−	−	+
6022	+	+	+	−	+	+	−	−	−
6023	+	+	+	−	+	+	+	−	−
6028	−	−	+	−	−	+	−	−	−
6029	+	−	+	−	+	+	+	+	−
6030	+	+	+	+	+	+	−	−	+
6031	+	+	+	−	±	±	−	−	−
6032	+	+	+	−	±	−	−	−	+
6034	+	+	+	−	+	+	+	+	−
6035	+	−	+	−	+	−	−	−	−
6043	+	+	+	−	−	−	−	+	−
6045	−	−	+	−	±	±	−	−	−
6047	+	±	+	−	+	−	−	−	−
6048	+	+	−	−	+	+	−	−	−

arterioles, arteries or sometimes in muscular arteries. In muscular arteries, fibrinoid material was usually deposited right beneath the endothelium, in media or adventitia or their combinations. The lesion was mostly observed in kidney, liver and heart.

Vascular lesions were found mostly in the lymph nodes, the edematous portion of the stomach and intestines. Of interest was case 6034, where whole layers of the ileum and large intestinal walls were hemorrhagic and necrotic. Here marked coagulation necrosis was seen in the mucosa, and most arterioles, capillaries and venules were plugged with fibrin thrombi. Many arterioles showed fibrinoid necrosis. Submucosal edema was apparent, and lymphatic fibrin thrombus formation was frequent (Fig. 6). In some arteries and arterioles neutrophilic infiltration was observed in the vascular walls, on which fibrinoid necrosis was apparent (Fig. 7). In case 6035, marked and extensive coagulation necrosis of lymph tissue was observed (Fig. 8). Many arteries in parenchyma and capsular connective tissue showed typical fibrinoid necrosis with moderately hyperplastic reticular element and infiltrated mononuclears surrounding the damaged vessels centripetally (Fig. 9).

Edema was usually found in the perivascular space throughout the brain.

Isolation and Identification of Hemolytic E. coli.

Attempts were made to isolate hemolytic *E. coli* from feces and small mesenteric lymph nodes of hogs that appeared clinically normal, collected from slaughterhouses. This experiment was performed to get a rough estimate of the incidence rate of this organism in local swine population. Hemolytic *E. coli* was isolated from about 16% (79/500) of the fecal samples examined, of which about 30% were isolated in almost pure culture. No organism was ever isolated from the mesenteric lymph nodes.

Thirteen litters of pigs, totalling 82 heads, in 5 farms were examined every 10 days for the presence of hemolytic *E. coli*. About 79% (65/82) of the pigs examined harboured this organism in their infancy. The mean time of first appearance of hemolytic *E. coli* in feces was about 60 days, ranging 39-97 days, after birth. The duration of bacterial inhabitation was 1-4 weeks. Occasionally, the organisms reappeared in the feces after various lengths of time. The data suggest that this organism is distributed widely among the local swine population. Only 1 out of 65 young pigs, however, was affected by ED during the period when hemolytic *E. coli* was demonstrable in their feces.

Sixty-six pigs with clinical signs of ED were obtained from Miaoli, Taoyuan, and Taipei Prefectures, and were autopsied. The results of hemolytic *E. coli* isolation are shown in Table VII.

From the table, it is apparent that hemolytic *E. coli* was present almost constantly in the intestinal contents of the diseased animals. Con-

TABLE VII

Hemolytic E. coli isolation from natural cases of edema disease of swine

Pig no.	Lung	Spleen	Liver	Mesenteric lymph nodes		Feces		Time of necropsy conducted
				Large	Small	Large	Small	
6129	—	—	—	—	—	—	—	D. 30 minutes
6006	—	—	—		—		—	Killed
6011	—	—	—		—		—	Killed
6033	—	—	—	—	—	—	—	D. T. 53 hrs
6060	—	+	+	—	—	—	—	Killed
6001	—	—	—		—		+	D. 30 hrs
6017	—	—	—	—	—	+	+	D. at PM room
6025	—	—	—	—	—	+	—	D. 6 hrs
6041	—	—	—	—	—	+	—	D. 2 hrs
6061	—	+	—	—	—	+	+	D. 20 hrs
6101	—	—	—	—	—	+	+	D. 17 hrs
6102	—	—	—	—	—	+	—	D. 9 hrs
6104	—	—	—	—	—	+	—	D. 2 hrs

TABLE VII *Continued*

Pig no.	Lung	Spleen	Liver	Mesenteric lymph nodes		Feces		Time of necropsy conducted
				Large	Small	Large	Small	
6134	—	—	—	—	—	+	—	D. 18 hrs
6135	—	—	—	—	—	+	+	D. 12 hrs
6137	—	—	—	—	—	+	+	D. 2 hrs
6143	—	—	—	—	—	+	+	D. 6 hrs
6144	—	—	—	—	—	+	+	D. 20 hrs
6146	—	—	—	—	—	+	—	D. 18 hrs
6169	—	—	—	—	—	+	+	D. 1 hr
6173	—	—	—	—	—	+	+	D. 5 hrs
6174	—	—	—	—	—	+	+	D. 10 hrs
6175	—	—	—	—	—	+	+	D. 2 hrs
6176	—	—	—	—	—	+	+	D. 2 hrs
6181	—	—	—	—	—	+	—	D. 6 hrs
6183				—	—	+	—	D. 6 hrs
6199	—	—	—	—	—	+	—	?
61101	—	—	—	—	—	+	+	D. 7 hrs
61102	—	—	—	—	—	+	+	D. 7 hrs
61104	—	—	—	—	—	+	+	D. 17 hrs
61109	—	—	—	—	—	+	+	D. 24 hrs
61110	—	—	—	—	—	+	+	D. 5-7 hrs
61112	—	—	—	—	—	+	+	D. 6 hrs
6005	—	—	—		—		+	Killed
6013	—	—	—	—	—	+	—	Killed
6016	—	—	—	—	—	+	—	Killed
6071	—	—	—	—	—	+	+	Killed
6083	—	—	—	—	—	+	—	Killed
6089	—	—	—	—	—	+	+	Killed
6099	—	—	—	—	—	+	—	Killed
6100	—	—	—	—	—	+	—	Killed
6130	—	—	—	—	—	+	—	Killed
6136	—	—	—	—	—	+	+	Killed
6009	+	+	+		+		—	D. 3 hrs
6073	—	—	—	—	+	—	—	D. 30 min
6007	+	—	—		+		—	Killed
6074	—	—	—	+	+	—	—	Killed
6012	—	—	—		+		+	D. 12 hrs
6020	—	—	—	+	+	+	+	D. 3 days
6037	—	—	—	—	+	+	+	D. 5 hrs
6039	—	—	—	—	+	+	+	D. 48 hrs
6088	—	—	—	+	+	+	+	D. 6 hrs
6105	—	—	—	+	+	+	+	D. 3 days

TABLE VII *Continued*

Pig no.	Lung	Spleen	Liver	Mesenteric lymph nodes		Feces		Time of necropsy conducted
				Large	Small	Large	Small	
6171	−	−	−	+	+	+	+	D. 14 hrs
6184	−	−	−	+	+	+	+	D. 4 hrs
61111	−	−	−	+	+	+	+	D. 4 hrs
61113	−	−	−	+	+	+	+	D. 6 hrs
6042	−	+	−	+	+	+	+	D. at PM room
6062	+	+	+	+	+	+	+	D. 15 hrs
6008	+	+	+		+		+	D. 27 hrs
6026	−	−	+	+	−	+	−	D. 10 hrs
6027	−	+	+	+	+	+	+	D. 12 hrs
6040	+	+	+	−	+	+	+	D. 48 hrs
61103	+	+	+	+	+	+	+	D. 17 hrs
6147						+	+	?
6148						+	+	?

D. Died after.
D. T. Died but received treatment with antibiotics before death.
? Exact time of death not available.

trarily, the organisms were consistently absent in mesenteric lymph nodes of the 10 sacrificed cases which gave positive intestinal cultures. Hemolytic *E. coli* was absent in the mesenteric lymph nodes of 28 out of 51 pigs died of ED.

When the organisms were present in the small intestines, the large intestines were also involved without exception. On the other hand, the reverse did not always hold true.

DISCUSSION

Seventeen cases of ED were successfully reproduced by inoculating supernatant of intestinal content collected from naturally occurring cases, confirming the observation of other investigators (1, 6, 8, 20-25). However, this is the first time that the identity of lesions between reproduced and natural cases has been confirmed by histopathology. Of interest were the vascular lesions found in pig 6035, killed at moribund stage 15 days after inoculation, in that fibrinoid necrosis of arterial walls was accompanied by periarteritis. The later lesion is similar to that of periarteritis nodosa, described by some authors as a lesion caused by hypersensitivity. It remains obscure whether this feature represented simple repair process of the damaged blood vessels, or alternatively, was caused by a specific type of inflammation of the blood vessels, including allergy. Pig 6034 lived for 5 days after inoculation, while the control animal died within 43 hours despite receiving 4.5 ml less inoculum than the former. This difference was obviously caused by treatment with antihistaminics in the former animal.

Many animals in our experiments died promptly with severe symptoms after inoculating intestinal contents. Timoney reported similar death of the experimental animals with histamine shock-like symptoms (1). Since the signs of intoxication can be inhibited by antihistamincs, there is no doubt that histamine was responsible for these signs. Histopathological similarity of lesions produced by histamine and aluminum hydroxide-gel-adsorbed-inoculum seems to support this conception. In our hands the ED producing-factor could be adsorbed by aluminum hydroxide-gel, but the supernatant of this adsorbed material, dialysed or non-dialysed, was still capable of producing signs of intoxication in experimental animals identical to those of natural cases. This is interpreted to mean that a substance other than the ED producing-factor was responsible for the intoxication signs. It appears unlikely that these intoxication signs were induced by exogenous histamine preformed in the intestinal content, because of the following considerations: (1) Histamine formed in the intestines is expected to be destroyed by the presence of histaminase in the gut; (2) histamine is removable by

dialysis, but our animals showed the same intoxication signs when they were inoculated with dialysed inoculum; and (3) the hemolytic *E. coli* strains examined did not produce decarboxylase. Alternatively, histamine of endogenous origin appears to be a better interpretation. This idea is agreeable with the recent observation of Greisman *et. al.* (31) that Gram-negative bacterial endotoxin exerts its toxicity through activation of a histamine-releasing factor in the blood.

Pigs inoculated with the supernatant of intestinal content could be protected from the first attack of intoxication symptoms by the simultaneous administration of antihistaminics. ED developed in these animals nevertheless, despite the fact that some of them appeared to survive longer, due perhaps to the use of antihistaminics. These findings seem to suggest that antihistaminics are only capable of relieving the intoxication symptoms caused by histamine in experimental animals, but incapable of suppressing the occurrence of ED, implying that histamine is probably not an essential factor in producing this disease. However, our unpublished data indicate that antihistaminics are therapeutically effective for natural cases of ED, in accordance with the results reported by Glattli (30). This may suggest that even though histamine is not an essential factor for initiating this disease, it may nevertheless play an important role in causing ED symptoms.

Pigs are highly sensitive to histamine. In this study a small dose of 2.4 mg/kg body weight proved lethal for this species within 5 minutes after injection, in contrast to the lethal doses for monkey of 50 mg/kg and for dog of 30 mg/kg (32). This may be the reason why the affected pigs terminated within a very short period after the onset of ED symptoms.

A surprisingly high incidence of hemolytic *E. coli* parasitism in grown hogs and young pigs was found in this study, similar to those reported by other workers (7, 19, 25, 27, 28). Organisms were isolated from 16% of hogs examined. Young pigs started to harbour this organism in intestines at about 2 months of age, and became free from organisms 1 to 4 weeks later. During this period, however, only 1 out of 65 hemolytic *E. coli* bearers exhibited symptoms of ED; this particular animal exhibited ED symptoms when all littermates yielded pure culture of hemolytic *E. coli* in the feces. In most instances hemolytic *E. coli* grew as a pure culture on the media, but the virulence of strains isolated from diseased and normal pigs was not compared. Nevertheless, the fact that hemolytic *E. coli* existed in many pigs without inducing ED indicates that for the production of this disease, some unknown co-acting factors are probably also involved.

Hemolytic *E. coli* was almost constantly isolated from intestinal contents of naturally occurring cases. Contrarily, this organism was never isolated from the mesenteric lymph nodes in 10 out of 12 slaughtered cases and in 28 out of 51 dead cases examined shortly after death. This finding is at variance with those reported by other investigators (1, 3-7, 12, 18). It is questionable whether what they dealt with represented mostly bacterial invasion from the intestine due to delayed examination.

Some authors (1, 3, 5, 6, 17, 29) claimed successful reproduction of ED by injecting frozen-thawed hemolytic *E. coli*, but none of our animals developed this disease when they were so treated. In this connection, case 6036 is interesting because of the presence of fibrinoid necrosis in a few medium-sized arteries: cavanous sinuses of the hypophyseal fossa in the sphenoid bone, interlobular arteries of the kidney, and one artery in the adrenal body. This animal was sacrificed 5 days after inoculation, and the correlation of these lesions with ED was obscure.

SUMMARY

Seventeen cases of ED were successfully reproduced by inoculating supernatant of the extract of intestinal contents collected from diseased pigs; the lesions produced were identical with those of natural cases, confirmed by histopathology. Attempts to reproduce ED by inoculating frozen-thawed hemolytic *E. coli* were always unsuccessful.

The active principles responsible for causing ED itself and intoxication symptoms seemed to be different chemical entities, because the former was removed by aluminum hydroxide-gel adsorption while the latter was not. Antihistaminics were effective in controlling the intoxication symptoms but not ED itself in experimentally reproduced cases.

Hemolytic *E. coli* was isolated from about 16% of 500 random fecal samples of hogs collected from slaughterhouses. Apparently normal young pigs started to carry this organism in their guts at about 2 months of age, but only 1 out of 65 hemolytic *E. coli* bearers exhibited symptoms of ED during this period. Hemolytic *E. coli* was the constant intestinal flora of naturally occurring cases of ED; however, this organism was isolated only with rarity from mesenteric lymph nodes of these cases.

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Fig. 1. Fibrinoid necrosis of a muscular artery in the adrenal body. Inoculated with hemolytic *E. coli* endotoxin. Case 6036. H. & E. Stain. 200×.

Fig. 2. Loosened arterial wall, presumably caused by edema, seen in the capsular connective tissue of the mesenteric lymph node. Inoculated with the supernatant of aluminum hydroxide-gel-adsorbed inoculum. Case 6075. H. & E. Stain. 320×.

Fig. 3. The changes are similar to Fig. 2. Inoculated with 200 mg of histamine phosphate. Died in 5 minutes. Case 6076. H. & E. stain. 320×.

Fig. 4. A venous thrombus found in the trabecula of a mesenteric lymph node. Note that the flattened and elongated cells, presumably endothelial cells, attached to the outer margin of the thrombus. This is caused by shrinkage of the tissue during processing. Died 5 minutes after the inoculation with histamine phosphate. Case 6076. H. & E. stain. 320×.

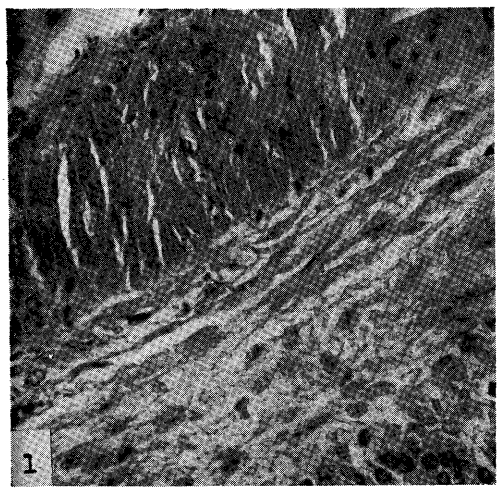
Fig. 5. Experimentally reproduced edema disease of swine. Marked edema seen in the submucosa of stomach. Inoculated with the supernatant of bowel extract.

Fig. 6. Fibrinoid necrosis of an arteriole and a lymphatic thrombus seen in the markedly edematous submucosa of intestine. Five days after inoculation with the bowel extract. Case 6034. H. & E. stain. 50×.

Fig. 7. Fibrinoid necrosis and neutrophilic infiltration in an artery at the edematous submucosa of intestine. A parietal fibrin thrombus is present. Moderate neutrophilic and mononuclear cell infiltration seen in the stroma of surrounding adipose tissue. Five days after inoculation with bowel extract. Case 6534. H. & E. stain. 200×.

Fig. 8. Extensive necrosis in the lymph node. Two arteries showed marked fibrinoid necrosis. A sleeve-like mononuclear infiltration seen in one artery. Fifteen days after inoculation with the bowel extract. Case 6035. H. & E. stain. 50×.

Fig. 9. Enlarged view of Fig. 8. Merked sleeve-like mononuclear infiltration around an artery, which showed typical fibrinoid necrosis, resembling periarteritis nodosa. H. & E. stain. 200×.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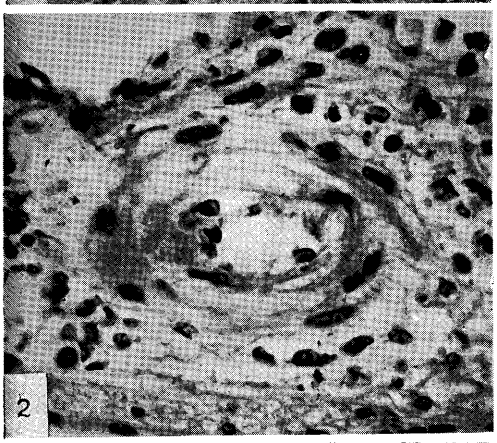
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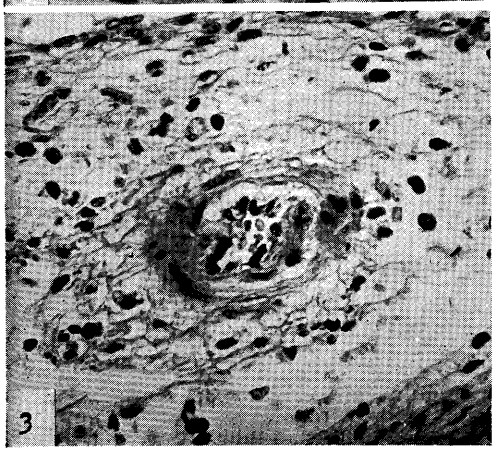
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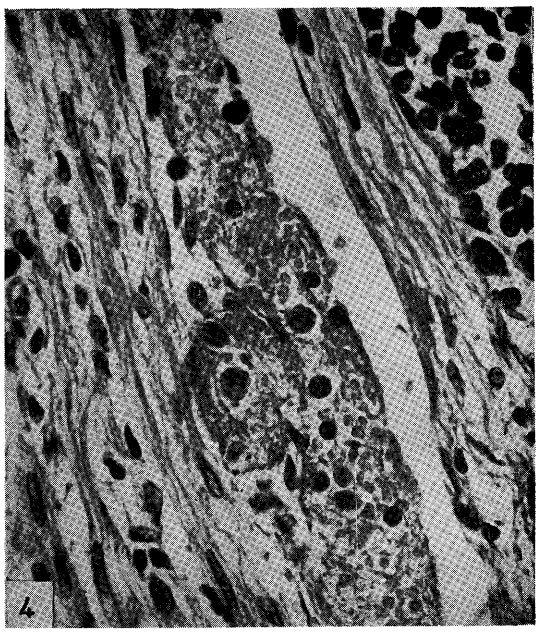
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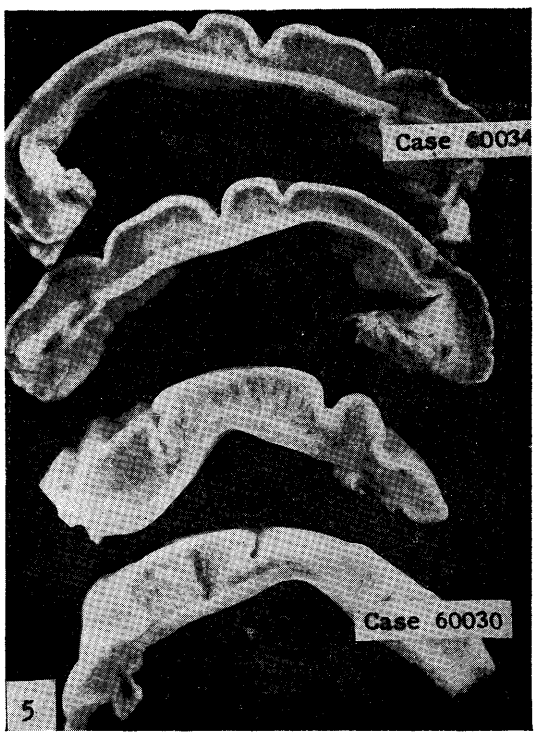

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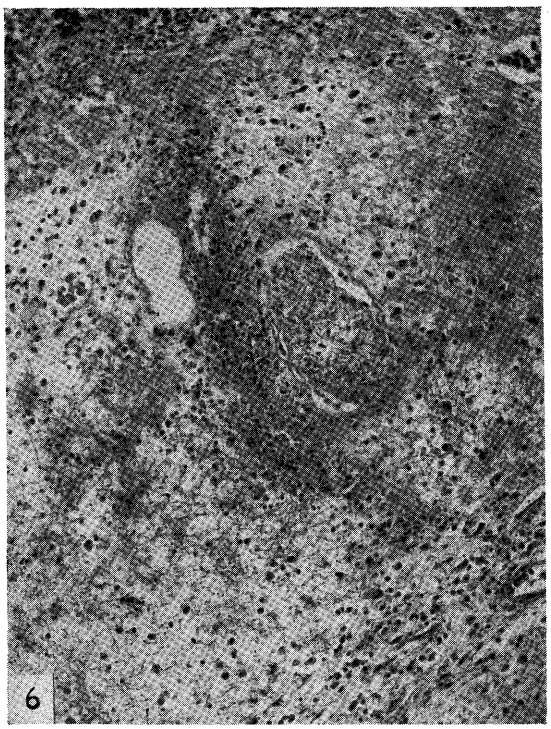
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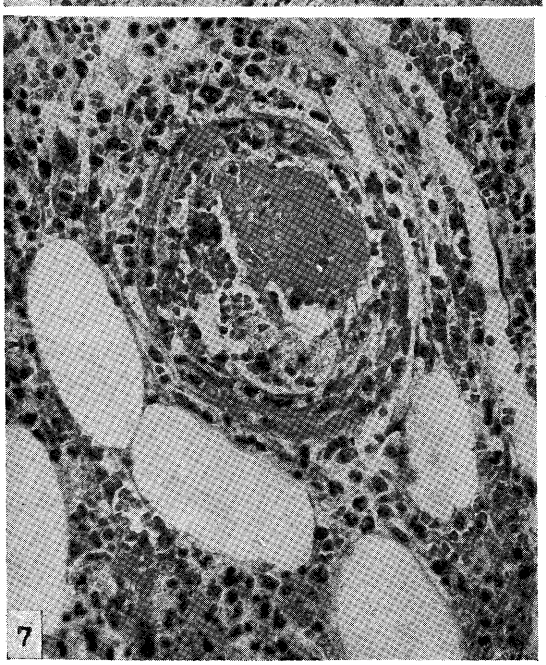
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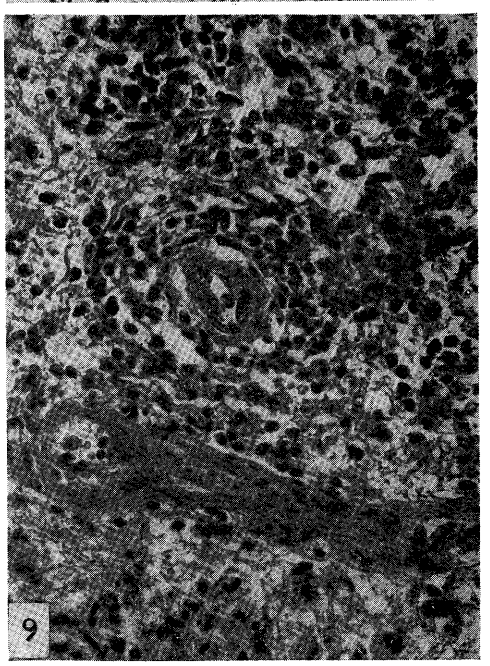
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