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Experimental Study on the Use of Homonymous Transplants of Esophagus in Dogs

By H. L. SKINNER, M.D., F.A.C.S.* JAMES K. CONN, M.D.* and J. F. OESTERLE, M.D.†

Resection of esophageal lesions has been a challenge to surgeons for more than 50 years. During that time, there have been numerous reports of successful resections. However, it has been only within the last decade that improvements in anesthesia and surgical techniques, along with the introduction of antibiotics, have permitted the achievement of acceptable mortality rates and end results. The diversity of proposed techniques indicates that an entirely satisfactory and standardized procedure has not yet been devised. A few of these may be mentioned. Resection with esophagogastrostomy is the usually accepted procedure and has been successfully performed for lesions as high as the cervical esophagus. However, the results of having a partial or complete thoracic stomach are not ideal, and a search has been under way continuously for an alternate method which can be used to reconstruct the esophageal continuity.

More and more successful attempts are being made to resect the lesion, whether carcinoma, benign tumor, or stricture, and to perform an esophago-esophageal anastomosis (*14*, *15*, *10*, *3*, *13*, *12*). That the blood supply of the thoracic esophagus is much better than had been thought has now been amply demonstrated. Parker and Brockington (*10*) resected 5–7 cm. segments of esophagus in dogs and anastomosed the cut ends. Their results remained good even when the entire thoracic esophagus had been freed from its bed prior to the resection and anastomosis. Swenson and Clatworthy (*15*) obtained good results with esophago-esophageal anastomosis in dogs after resecting 16 to 80 percent of the esophagus. More recently, MacManus, Dameron, and Paine (*7*) have performed experimental studies showing that the blood supply of the esophagus in dogs is not jeopardized by freeing the entire thoracic esophagus as long as

*Chief of Surgery, and Senior Assistant Surgeon, respectively, U. S. Marine Hospital, Staten Island, N. Y., Public Health Service.

†Outpatient Dispensary, Washington, D. C., Public Health Service.

the cervical portion remains intact. Skinner and Lloyd (*12*) in their case report have shown that the entire esophagus can be mobilized with secondary resection and end-to-end anastomosis. Because of the inadequacy of certain aspects of all the procedures, various modifications have been proposed including a tubed pedicle skin graft to replace the esophagus (1), lung-pleura graft to protect the suture line (*2*), and pedicle lung flap to strengthen a weakened area of the esophagus (*9*).

In view of the above advances and the success in transplanting many of the body tissues, the question arose as to whether it would be possible to perform esophageal transplants. The problems involved were similar to those encountered in performing arterial grafts, so the methods used by Peirce, et. al. (*11*), and Gross, Bill and Peirce (*4*) for preserving arterial segments were adapted for our use in preserving esophageal grafts.

Method

Homonymous transplants were performed in 20 dogs. No attempt was made to use any particular breed, but a uniform size of 20 to 30 pounds was maintained insofar as possible. A self-perpetuating system for supplying the grafts was established whereby a segment was removed from one animal, preserved for varying lengths of time, and transplanted into another animal which supplied the next graft. The size of the graft varied from 3 to 7 cm. in length. It was observed that these segments shrank following their resection to about 75 percent their size in situ. They did not change further in size during the period of preservation.

The preservative was that used in studies on aortic grants (*4*, *11*), 90 percent balanced salt solution and 10 percent serum. Balanced salt solution is prepared as a stock solution [1] and diluted five times prior to use. A buffer solution of 1.4 percent $NaHCO_3$ is prepared, stored separately, and added to the BSS in a ratio of 2.5 cc. to 100 cc. at the time of use. Equilibrium of the system is at pH 7.6.

The transplants were placed in the previously autoclaved solution as soon as they were removed from the animal; 100,000 units of penicillin and 1.0 gm. of streptomycin were added, and within 2 hours serum was added. The serum was obtained from blood taken from the azygos vein at the time of operation. The flasks were covered with layers of gauze and rubber sheeting and placed in an ordinary refrigerator until ready for use. We felt that addition of the antibiotics reduced the activity of normal esophageal flora and also minimized the risk from chance contamination. The majority of the transplants were preserved for 7 days. However, in five instances,

[1] Formula for stock solution BSS: H_2O 250 cc.; NaCl 20 gm., KCl 1-0 gm., $MgCl_2$-6 H_2O 0.2 gm., $CaCl_2$ 0.35 gm., Na_2HPO_4 0.15 gm., KH_2PO_4 0.15 gm., glucose 2.5 gm., 0.4 percent phenol red 12.5 cc., 1 cc. chloroform, 0.36 gm. Na_2HPO_4-12 H_2O dissolved separately and added to solution.

the length of preservation was 1, 6, 8, 13, and 14 days, and two grafts were transplanted immediately without preservation.

Anesthesia was provided by a single I. V. injection of sodium pentobarbital in a dose of 1.0 gr. per 5 lbs. body weight. Artificial respiration was maintained by means of an automatic respirator,[2] with a continuous flow of oxygen, connected to an endotracheal tube. The surgery was carried out through a right postero-lateral incision. The pleural space was entered through the bed of the sixth rib, although, occasionally, the fifth was used with equal satisfaction. A Tuffier retractor provided excellent exposure through this incision. The collapsed right lung was packed anteriorly with moist sponges. The mediastinal pleura was incised over the segment of esophagus to be resected from the azygos vein for a distance of approximately 10 cm. distally. An attempt was made to preserve intact flaps of mediastinal pleura to facilitate subsequent closure. The above portion of esophagus was then freed from its bed, the few vessels entering it being ligated and divided as close to the wall as possible. Care was exercised in handling the esophagus to avoid crushing or otherwise injuring the wall. Traction sutures were placed in the wall about 10 cm. apart and the intervening segment, varying from 5 to 8 cm. in length, was excised. The transplant was then placed in the defect and proximal and distal anastomoses performed.

Anastomosis was in two layers, the mucosa being approximated with simple interrupted sutures of No. 0000 chromic intestinal catgut and the muscle, with simple interrupted sutures of No. 000 intestinal cotton. As few sutures as possible were used, approximately 8 to 12 being placed in each layer. No attempt was made to produce a water-tight closure, and no signs of leakage at the suture lines were noted. The mediastinal pleura was closed, the lung reexpanded, and the chest wall closed in layers without drainage. We found this simple method of anastomosis to be most satisfactory. Methods employing continuous suture, or those producing inversion or eversion of the edges, tended to cause stenosis at the suture line.

Postoperatively, the animals received 1 injection of 300,000 units of repository penicillin. For 48 hours they were given nothing by mouth but received hypodermoclysis of 250 cc. of 5 percent glucose in saline daily. On the third day they were given 250 cc. of milk and water twice. Following this, Pablum and strained and chopped foods were added to the diet as tolerated.

Results

Of 20 dogs operated upon, 11 survived longer than 1 week. Of the 9 that died, 5 deaths were due to necrosis of the grafts with disruption of 1 of the suture lines. The four remaining deaths, while not due to

[2] Rand respirator.

necrosis of the transplant, occurred because of the operative procedure and are included for the sake of completeness. One death was attributed to empyema which followed massive soiling of the pleural space by regurgitated gastric contents while the esophagus was open. Another was due to massive hemorrhagic pleural effusion. A third failed to resume normal respirations when the respirator was discontinued, and the last one died of cardiac arrest while the esophagus was being dissected free. One other developed cardiac arrhythmia and cessation of beat during the procedure but responded to application of 1 percent procaine to the region of the pacemaker and to cardiac massage. Three microscopic examinations of the tissues were made of (1) the fresh specimen prior to preservation, (2) the preserved transplant, and (3) the specimen at the time the animal was sacrificed.

Total grafts performed	20
Surviving more than 1 week	11
Grafts sloughed	5
Other deaths	4
Gastric spillage with massive pleural infection	1
Anesthetic death	1
Cardiac arrest	1
Massive pleural effusion	1

The animals were sacrificed when it became impossible to maintain satisfactory nutrition by the oral route. No attempt was made to prolong life by parenteral feedings.

The dogs that survived the immediate postoperative period followed a characteristic course and exhibited similar end results. They were uniformly active and took fluids well for 1 to 2 weeks. The majority of the animals tolerated strained foods well during the second week but occasionally vomited. The process of stenosis began about the third week and became progressively more severe until practically anything taken by mouth was regurgitated. As stenosis became complete, even accumulations of saliva were vomited from time to time. The speed with which stenosis progressed varied from animal to animal and is thought to represent merely individual tissue variation. Only one animal had hematemesis, and this was transient.

At autopsy, the grafted area was found to be densely attached to the surrounding structures by appreciable vascular adhesions. The anastomotic lines were firmly healed. The graft was thickened and very firm and was generally contracted toward the center. The lumina varied from 1–2 mm. to 0.5 cm. in diameter. When the specimen was opened longitudinally, a small area of stenosis was seen at the center of the graft. Fresh mucosa had grown in toward the middle from the ends. This picture was found irrespective of whether the graft was transplanted in the fresh state or had been preserved for a period of time up to 14 days.

Results of homonymous grafts performed on dogs

Dog	Weight	Date	Graft from	Size graft (cm.)	Length of preservation (days)	Course	Survival (days)	Result
B	18	12/22/49	A	3	7	Fluids and soft foods first 2 weeks. Occasional emesis third week. Never any solid food. Constant emesis by fifth week.	50	Sacrificed, weight 10 pounds. Almost complete stenosis at distal anastomosis.
C	22	12/29/49	B	3	7	Extensive spillage of gastric contents at operation.	7	Pneumonic consolidation on right. Proximal suture line sloughed. Graft partially necrotic.
D	22	1/5/50	C	3	7	At operation graft appeared poorly preserved. Was placed in BSS while hot.	2	Graft necrotic.
E	20	2/12/50	D	3	7	First week active and well. Took liquids. Second week took strained foods but vomited chopped foods. Third week tolerated only liquids and showed hematemesis.	27	Sacrificed, weight 14 pounds. Graft thick and generally constricted with stenosis at center.
F	14	1/19/50	E	3	7	Active and well. Took liquids and strained foods for 2 weeks. Failed sharply third week. Vomited saliva and all oral intake.	22	Sacrificed, weight 10 pounds. Complete stenosis with contraction in all dimensions.
G	24	1/26/50	F	5	7	Failed to react from anesthesia, possibly because of obstruction of trachea by mucous.	-------	
H	20	2/3/50	G	5	8	Did well for 2 weeks. Took milk and ground food. No emesis until third week.	23	Stricture at center graft. Dog died. Weight 18 pounds.
I*	20	2/10/50	-------	-------	-------	-------	-------	
J	22	2/16/50	H	5	13	Died after 12 hours. At necropsy there was a massive hemorrhage pleural effusion. Neither lung was aerated. Graft was intact.		
K	18	2/17/50	I	5	7	Died on third day	3	Graft necrotic. Suture line sloughed.
L	16	3/2/50	J	4	14	Usual course of progressive improvement for 2 weeks followed by increasing stenosis.	18	Sacrificed, weight 10 pounds. Complete stenosis.
M	28	3/3/50	L	4	1	Same	17	Sacrificed, weight 20 pounds. Complete stenosis.
N	28	3/9/50	M	4	6	Normal postoperative course until the fifth day when febrile reaction set in.	6	Pneumonia on right. Necrosis of graft with partial slough.
S	-------	4/13/50	N	4	7	Poor course	4	Died. Graft necrotic.
U	-------	4/27/50	V	4	(1)	Normal course. Dilated bid but these not as satisfactory as in previous cases. Showed symptoms of progressive stenosis.	21	Sacrificed. Almost complete stenosis.
V	-------	4/27/50	U	4	(1)	----do	28	Do.

*No graft performed. Animal died in cardiac arrest while esophagus was being exposed.
[1] None, immediate transplant.

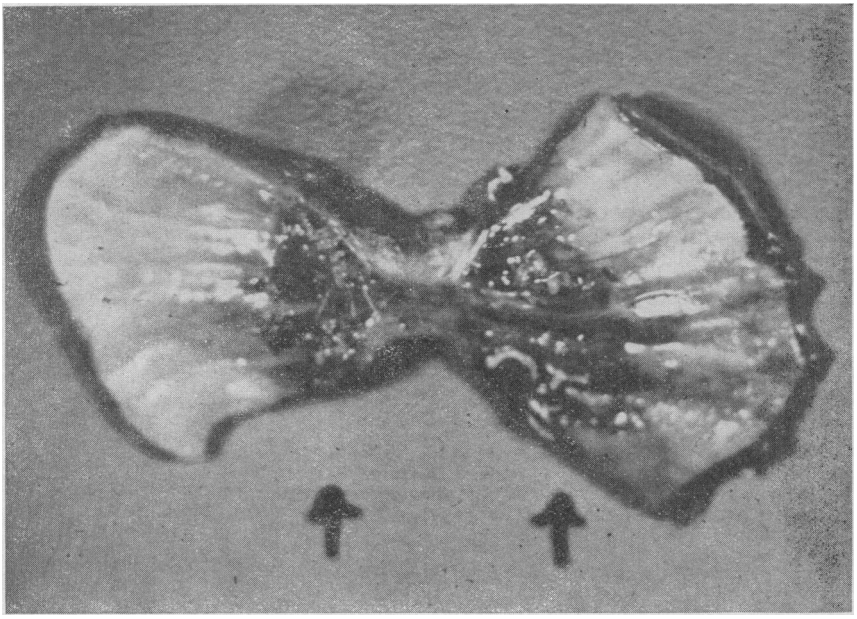

Figure 1. Typical graft when not dilated. This specimen is 27 days postoperative. Note the complete stenosis at the center of the graft.

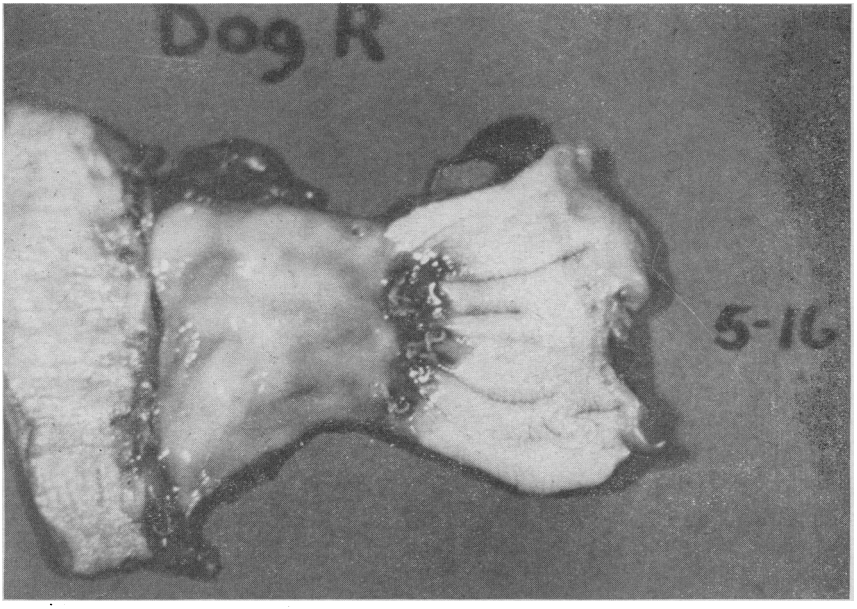

Figure 2. Homonymous graft 40 days postoperative. Stenosis minimal, mucosa is reepithelialized. Proximal suture line was inadvertently cut in removing specimen.

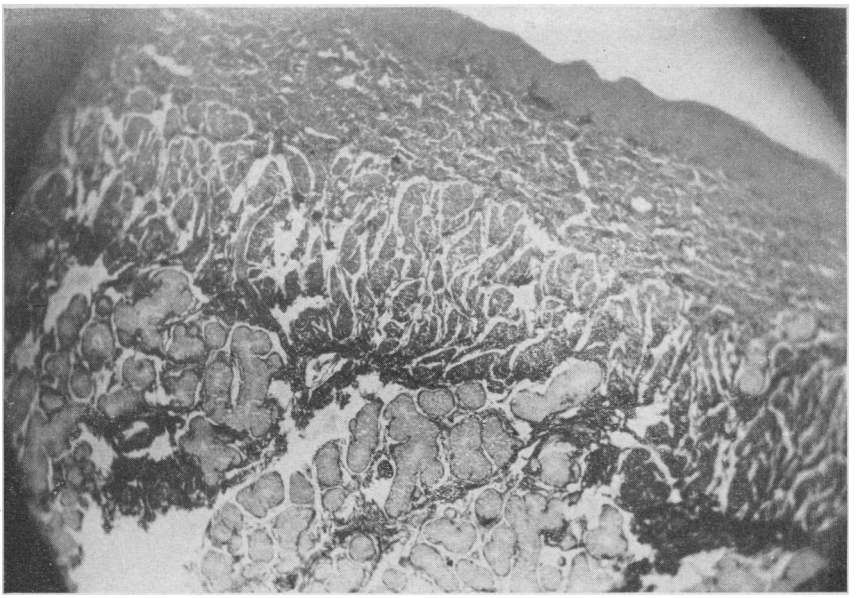

Figure 3. Portion of normal esophageal wall showing mucosa, submucosa, one muscle layer, and mucous glands which are abundant in dogs.

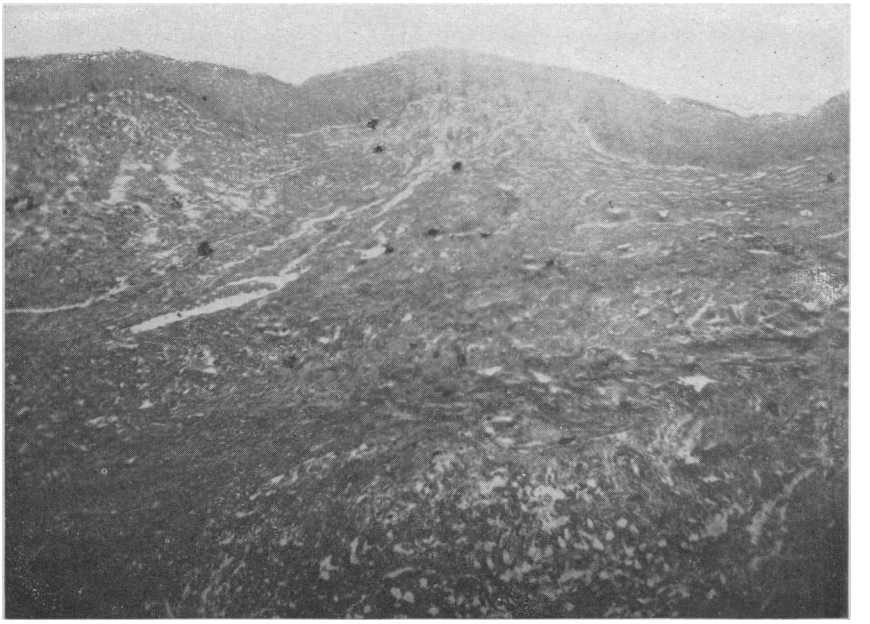

Figure 4. Portion of wall of graft showing replacement of all elements by fibrous tissue. Note that fresh mucosa is present in this portion of graft.

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Microscopically,[3] the tissue after preservation exhibited histological integrity. There was minimal evidence of edema and hydrops and some change in staining characteristics of the glands. The gross impression of the autopsy specimen was confirmed by microscopic examination. There was new mucosa at the ends of the graft with an ulcerated central area. The wall was degenerated with fibroblastic replacement of much of the muscle, although some smooth muscle bundles were intact.

Because of these findings, we felt that patency of the drafts might be preserved by repeated dilitation. This process was tried on five dogs, O, Q, R, U, and V. A No. 24 esophageal dilator was used, but mechanical difficulties permitted satisfactory dilitations only in dog R. The dilator used was not large enough for complete dilitation and was not rigid enough to withstand constant use. Passage of the dilator did not appear to cause the animal any particular discomfort. No anesthesia was required, and the only untoward effect noted was a gagging reaction. We found that, to be effective, dilitations should be instituted on the 7th postoperative day and should be done twice daily. Each dilitation resulted in markedly improved function for 6 to 8 hours. Dilitations could not be performed with complete satisfaction in dogs O, Q, U and V and stenosis developed progressively. However, they exhibited a better course than those which were not dilated in that there was less vomiting and nutrition was better maintained. The dilitations were completely satisfactory in dog R. This animal tolerated kennel rations in small and frequent feedings. A barium swallow, performed on the 23d day, showed some stenosis with proximal dilitation. Death which occurred on the 40th day was due to distemper. The autopsy specimen presented an entirely different picture than the others. The wall of the graft was thin and pliable, the lumen being only slightly reduced in size. No stenotic area was present. The surface was completely reepithelialized. Microscopically, this specimen showed essentially the same changes as the others except that the mucosa was completely regenerated and there was less cellular infiltration.

A few conclusions can be drawn from this limited preliminary study. Esophageal tissue will remain viable when refrigerated for a period as long as 14 days in a mixture of 90 percent balanced salt solution, 10 percent serum, streptomycin, and penicillin. This tissue apparently remains alive when transplanted to another animal of the same species. Revascularization seems to take place slowly from the esophageal bed and surrounding tissues. In the meantime, degeneration of the muscular elements, glands, and mucosa is possibly caused by ischemia, and replacement of fibrous tissue takes place with its resultant

[3] Histological specimens reviewed by Lawrence H. Sophian, Medical Director, Public Health Service, Chief of Pathology, U. S. Marine Hospital, Staten Island, N. Y.

contraction and stenosis of the lumen. We believe that an inference can be drawn from this work, namely, that a functional graft can be obtained if patency is maintained until the scarring process ceases. If such an inference proves to be correct, then further studies are in order to determine the maximum amount of esophagus which can be removed and replaced by a graft.

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Dried Smallpox Vaccine

By J. W. Hornibrook, M.D. and W. H. Gebhard, B.S.*

The advantages of a stable smallpox vaccine are easily apparent. With such a product, effective vaccination would be possible even in places without adequate refrigeration. Storage and shipment would be simplified, and the cost of recalling and replacing outdated material would be reduced.

In 1927, Otten (*1*) reviewed the history of dried vaccine production and reported on its successful use in Batavia. He emphasized the importance of sealing the vaccine under a high vacuum. The results of 16,000 vaccinations performed with dried vaccine derived from buffalo source were summarized by Otten in 1932–33 (*2*). This vaccine remained stable for days at 58° C., for months at 37° to 42° C., and for a year at average room temperature in the tropics. In 1943, Morozov et al. (*3*) reported on a dried vaccine which was still considered potent at a 1/1000 dilution after storage for 30 to 35 days at 37° C. In 1949, Nagano (*4*) reported on the use of dried vaccine which compared favorably with a liquid vaccine when tested in persons.

Three major difficulties have been encountered in the preparation and use of dried smallpox vaccine, and these probably account for its not being more widely used. First, it has been difficult to obtain preparations in which the numbers of contaminating bacteria have been reduced to an acceptable level. Second, it is more difficult to reconstitute a dried vaccine at the time of use than to open a capillary of liquid vaccine. Third, it is more expensive to produce dried vaccine than it is to produce the liquid product.

The study described in this report was undertaken to investigate ways of reducing or eliminating the disadvantages of dried preparations. In addition, it was thought worth while to investigate the drying of calf vaccine. Otten used a vaccine derived from buffalo.

Method

A laboratory, licensed for smallpox vaccine production by the National Institutes of Health, prepared the calf-derived vaccine pulp, lot. No. 690, following the method described by Ducor (*5*). After a sample was taken for bacterial count determinations and

*Laboratory of Biologics Control, Microbiological Institute, National Institutes of Health, Public Health Service; and Division of Laboratories, Michigan Department of Health, respectively.

safety tests, the pulp was suspended in a lactose salt mixture (*6*),[1] a weight volume ratio of one part pulp to three parts menstruum.

The pulp was ground in a Waring Blendor for 50 minutes. To prevent overheating the mixture, the total grinding time was divided into five 10-minute operations, and the mixture was refrigerated at 4°–6° C. until cold after each operation. An aqueous solution of crystaline penicillin G was added to the pulp suspension to make a concentration of 10 units per ml. at the conclusion of the fourth grinding period.

Using a capillary pipette, 0.5 ml. amounts of the pulp suspension were dispensed into sterile, cotton-plugged, pyrex tubes 8 by 110 mm. The inner walls above the vaccine level in the tubes were cleaned with sterile cotton swabs to prevent charring of residual vaccine during subsequent flame sealing.

To facilitate individual vaccination, the vaccine was made to adhere to the vaccination needle by the following method: steel vaccine needles, roughened with an emery wheel for a distance of one-fourth inch from the tip of the needle were dipped in 10-percent human albumin solution and allowed to dry at room temperature. Half-hitches of No. 25 linen thread were cast on to the roughened surface to form a linen surface approximately one-fourth inch in length. Each needle was then placed in a 7 by 95 mm. soft-glass tube that had previously been constricted in the middle to a diameter of about 3 mm. The tubes were plugged with nonabsorbent cotton and autoclaved for 20 minutes at 20 pounds steam pressure to sterilize the needles and to fix the thread firmly onto the needle by coagulating the albumin. When the tubes had cooled, one drop of vaccine was introduced by capillary pipette into each tube, the tube being manipulated so that the vaccine was brought into contact with the linen surface of the needle.

The method described for the preparation of vaccine-impregnated needles might be economically adapted to routine procedures if small, thin-walled vials were used and an automatic thread wrapping machine can be devised, or if other absorptive materials similarly located on the needle are used.

Two drying methods were used:

1. After freezing the vaccine at −18° C., the cotton plugs were removed from some of the tubes. They were then attached to the manifold of a sulfuric acid drying apparatus (*7*) which was then evacuated to a pressure of 50 microns of mercury. After 20 hours, the

[1] This mixture had the following composition: potassium citrate ($K_3C_6H_5O_7 \cdot H_2O$) 1.35 gm., sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) 2.45 gm., potassium phosphate (K_2HPO_4) 0.61 gm., calcium chloride ($CaCl_2$ anhydrous) 1.33 gm., magnesium chloride ($MgCl_2 \cdot 6H_2O$) 0.6 gm., potassium carbonate ($K_2CO_3 \cdot 1\frac{1}{2}\ H_2O$) 1.0 gm., lactose 57.5 gm., lactic acid q. s. to pH 7.00 (about 0.65 cc.), H_2O 1,000 cc. Dissolve all ingredients except $CaCl_2$ in 500 cc. water. Dissolve $CaCl_2$ in 500 cc. water. Mix the two solutions and adjust pH. Filter through a Berkfeld filter.

tubes were sealed at the constriction while still attached to the manifold under a maximum pressure of 50 microns of mercury.

2. The remainder of the tubes, plugged with cotton, were placed in 500 cc. bottles and attached to a 1-liter glass condenser. The latter was immersed in an insulated container of dry ice (solid CO_2) and alcohol. Connecting tubes between the condenser and bottles were at no place less than one-half inch in diameter. The pressure was then reduced to 50 microns of mercury. After 48 hours, the tubes of vaccine were removed, attached to a manifold, and sealed under a pressure of 50 microns.

Results

Bacterial Counts

The National Institutes of Health mininum requirements for smallpox vaccine state that the bacterial count on the final vaccine shall not exceed 1,000 organisms per ml. Determinations on the frozen pulp, sampled before it was suspended in the lactose salt menstruum, gave an average of 14,000 organisms per gram of pulp. After dilution with the lactose salt solution, addition of penicillin, and drying, the count of the reconstituted vaccine dropped to an average of 600 organisms per ml., well below the maximum number allowed.

Potency Tests

1. Vaccines prepared by drying methods 1 and 2 gave essentially similar results:

> Method 1—80 percent confluency at the 1:1,000 level, and 7 vesicles at the 1:10,000 level.
>
> Method 2—50 percent confluency at the 1:1,000 level, and 9 vesicles at the 1:10,000 level.

This vaccine, when stored at 37° C. for 43 days, gave 7 pocks at 1:1,000 and 3 pocks at 1:3,000. After 113 days at 37°, we obtained confluency at a 1/10 dilution, 13 pocks at 1/100, and 7 pocks at 1/1000.

Another lot of dried vaccine from the same source and of relatively the same potency gave a 50 percent confluent reaction at 1:100 after being held at 37° C. for 80 days. The above potency testing was done by the method described in the Smallpox Minimum Requirements of the National Institutes of Health. The vaccines were all reconstituted to original volume before diluting.

2. The individually wrapped vaccine needles were tested on rabbits by rotating the vaccine-saturated thread consecutively in drops of water placed on five sites on the rabbit. The needle was then used by the multiple puncture method to vaccinate in these five places. Vaccinial vesicles were obtained in the first four sites in one instance and in all five in another.

3. Twelve individuals were vaccinated with the dried vaccine reconstituted to original volume in distilled water. This vaccine had been stored at 4°–6° C. Of four previously vaccinated persons three had accelerated reactions and one an immune reaction. Four persons not previously vaccinated had primary reactions. Of four persons with questionable histories of vaccination, one had a primary reaction, one an accelerated reaction, and two had immune reactions.

Five individuals who had been previously vaccinated were vaccinated with the dried vaccine reconstituted to four times the original volume in 0.5 percent phenol after storage for 43 days at 37° C. Only 10 insertions were made per person. In this group there were three immune reactions, one accelerated reaction, and one negative reaction.

4. A tube of vaccine, reconstituted to the original volume with 0.5 percent phenol and stored 24 hours at 4°–6° C., gave 100 percent confluency at a dilution of 1:100 and 80 percent confluency at a dilution of 1:1000 when titrated. The same material stored 24 hours at room temperature (26.6° C.) gave 90 percent confluency at 1:100 and 3 vesicles at 1:1000. This decrease in potency after standing makes it advisable to use the vaccine on the day of reconstitution.

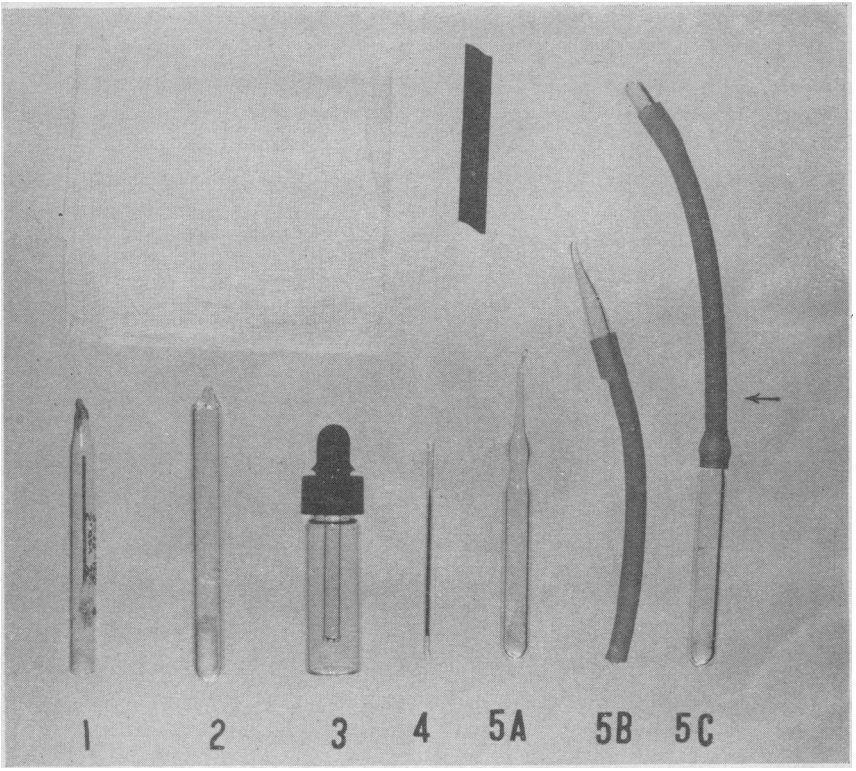
Methods of Reconstitution of Dried Vaccine

A major problem in the use of a dried vaccine is a convenient and safe method of reconstitution and administration. A definite hazard is involved in opening the vials of vaccine because there is a tendency for a small amount of the powdered pulp to be blown from the evacuated tube when it is broken. This pulp could cause an ocular or respiratory infection, or a deep vaccinia infection might be produced from accidental cuts on broken glass.

Several methods have been devised to overcome these difficulties. For individual vaccination, the arrangement shown by No. 1 in the illustration can be used. The ampoule is scratched with the file, wrapped with the sterile gauze, and broken. A drop of 0.5 percent phenol in water is placed on the site of vaccination by means of the dropping bottle (No. 3). The linen wrapped portion of the needle is then rotated in the drop of diluent, and the patient is vaccinated through this drop by means of the needle point.

When several persons are to be vaccinated at one time, the vial containing the vaccine pellet (No. 2) can be scratched with the file, wrapped with gauze, and broken. The pellet of vaccine can then be dropped into the bottle (No. 3) which contains 0.5 percent phenol. The cap is replaced and the bottle shaken to suspend the vaccine.

Perhaps the safest method is that shown in Nos. 5A–C. A thin-walled rubber tube sealed at one end and containing 0.6 ml. of 0.5 percent phenol is slipped over the neck of the ampoule. When the tip of



Methods for reconstituting and administering dried vaccine.

the ampoule is broken by bending at the arrow (No. 5C), the vacuum causes the solution to enter the vial and reconstitute the vaccine. The vaccine may then be removed by dipping a wooden applicator into the solution and applying it to the skin. Vaccination is then done in the usual way with the needle.

Summary

1. Smallpox vaccine prepared from calves can be dried with only slight loss of potency. Simple equipment will serve in the drying process.

2. If penicillin is added to a carefully prepared vaccine before drying, the minimum requirements as to bacterial count can be satisfied.

3. Several methods for packaging and reconstituting the dried vaccine in convenient form are described.

4. The hazards of reconstituting dried vaccine are discussed.

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Inhibition of a Strain of *Brucella abortus* By Medium Filtered Through Cotton

By Donald M. Boyd and Ezra P. Casman*

The study of cotton as a possible source of factors inhibitory for the growth of *Brucella abortus* was suggested by observations made at the Department of Agriculture's Animal Disease Station at Beltsville, Md. It was noted there that a potato-infusion agar medium clarified by centrifugation gave excellent growth of *Br. abortus*, whereas the same medium filtered through cotton was inferior in supporting growth of the organism.

In order to determine the nature of the inhibition resulting from filtration through cotton, the effect of this procedure on the growth of a fastidious strain of *Br. abortus* on a simple and reproducible medium was studied.

Materials and Methods

The test organism employed in the experiments described below was strain No. 2451, isolated July 1947 from the milk of an infected cow at the Animal Disease Station of Beltsville, Md. It required at least 5 days of incubation in an atmosphere of 10 percent carbon dioxide for the development of appreciable growth on solid media.

In preliminary experiments it was found that tryptose agar (Bacto) was incapable of supporting growth when inoculated with very dilute suspensions of the test organism. Since this deficiency could be removed by adding starch to the medium, the removal of fatty acids seemed advisable in view of the work of Ley and Mueller (*1*). Removal of fatty acids by extracting acidified tryptose (Bacto) with ether and by washing the agar with large amounts of alkaline (pH 9.0) distilled water, using the technique of Casman (*2*), rendered the medium capable of supporting growth of the test organism. Since these purification procedures were time consuming and gave variable results, a simpler and more satisfactory solution to the problem was obtained by modifying the medium through addition of the minimal amount of starch (0.03 percent) necessary for the neutralization of the fatty-acid effect. The basic medium was prepared according to the following formula:

	Grams
Agar (Bacto)	15
Tryptose (Bacto)	20
NaCl	5
Dextrose	1
Corn starch	0.3

Cold distilled water to make one liter; pH 6.8.

* Food and Drug Administration, Federal Security Agency.

The mixture was shaken while cool to disperse the starch and heated to boiling. After autoclaving at 121° C. for 15 minutes, it was cooled and distributed in sterile Petri dishes provided with porcelain tops, the outsides of which were glazed.

The plates were streaked uniformly with very light saline suspensions (approximately 100,000 organisms per ml.) of the *Brucella* culture. A one mm. loopful of the suspension was placed at the edge of the medium and the inoculum was spread by streaking with the same loop in such a manner as to permit determination of the ability of the medium to support the growth of both isolated and large concentrations of organisms. The plates were incubated at 37° C. under 10 percent carbon dioxide, and examined at intervals between the 3d and 10th days for distribution and amount of growth. Duplicate plates were streaked, and inhibition was determined by comparison with control plates. Inhibition varied in degree from decrease in the size of the isolated colonies, through absence of discrete growth, and, finally, complete absence of growth in both lightly and heavily inoculated portions of the plates.

Results

The effect of filtration of the basic medium through each of 15 different commercial brands of absorbent cotton was first studied. One-hundred-ml. quantities of the hot medium were filtered through 10-gram portions of the cotton samples and the filtrates were compared with a portion of the medium which was not filtered. Although the latter supported good growth of the test organism, all 15 of the filtered portions showed no growth after 7 days of incubation.

An attempt was next made to determine whether filtration through cotton removed an essential ingredient from the medium or resulted in the addition of a growth-inhibiting substance. Five hundred ml. of the basic medium were filtered while hot through 10 grams of absorbent cotton. A second 500-ml. portion was prepared with distilled water which had been filtered while hot through another 10-gram portion of the cotton and a third portion of the basic medium was prepared without any filtration of the medium or its water. Growth on the control unfiltered medium became visible to the naked eye on the 4th day of incubation and was excellent on the 10th day. The first and second media were unable to support the growth of the test organism.

Having found that the cotton was the source of a growth-inhibiting substance, experiments were conducted to determine the amount and nature of this material.

When 200 ml. of the hot basic medium were filtered through 8.5 grams of absorbent cotton and diluted serially with unfiltered medium,

complete inhibition of growth was obtained in the 1:4 dilution and partial inhibition in the 1:8 dilution (table 1).

Table 1. *Growth of* Br. abortus *No. 2451 on cotton-filtered medium diluted with unfiltered medium*

Incubation (*days*)	Dilutions					Control
	1:2	1:4	1:8	1:16	1:32	
3	0	0	0	0	±	±
4	0	0	±	±	1	1
5	0	0	±	1	1. 5	1. 5
6	0	0	1	2	2	2
10	0	0	2	3. 5	4	4

Key: 0=none; ± = trace; 1=distinct; 2=moderate; 3=good; 4=excellent.

Since the inhibitory agent contributed by filtration through cotton could be neutralized by increasing the starch content of the medium to 0.1 percent, and since the cotton was found by Soxhlet extraction to contain from 0.4 to 0.6 percent ether-soluble material, inhibition by fatty acids was again indicated.

The fatty-acid content of a hot-water extract of cotton was removed by ether extraction, and the ether extract and ether-extracted water were examined for inhibitory activity according to the following technique: Ten grams of cotton were extracted with 500 ml. of hot distilled water, the extract adjusted to pH 2.0 with tenth-normal HCl and extracted three times in a separatory funnel using two volumes of ether for each extraction. The dried ether extract weighed 12 mg., representing 0.12 percent of the weight of the cotton. The extract was added to the basic unfiltered medium prior to autoclaving to give various concentrations. Complete inhibition of growth was obtained with a 1-mg. percent concentration (10 mg. per liter). When the ether-extracted water was readjusted to pH 7.0 with tenth-normal NaOH and used in preparing the basic medium, growth was as good as that on the control basic medium (see fig. 1).

The mean molecular weight of the fatty acids removed from cotton was determined by the following method: One kilogram of absorbent cotton was extracted with approximately 5 liters of boiling water and the extract was concentrated to 500 ml. by boiling. The neutral fats were removed by adjusting the concentrate to pH 12 with N/10 NaOH in the cold, adding an excess (2 ml.) of N/1 NaOH and extracting three times, using two volumes of ether for each extraction.[1] The extracted water was then adjusted to pH 2.0 with N/10 HCl, and the free fatty acids were removed by extracting three times

[1] This procedure permitted the conversion of free fatty acids to their soaps, without saponifying the neutral oils.

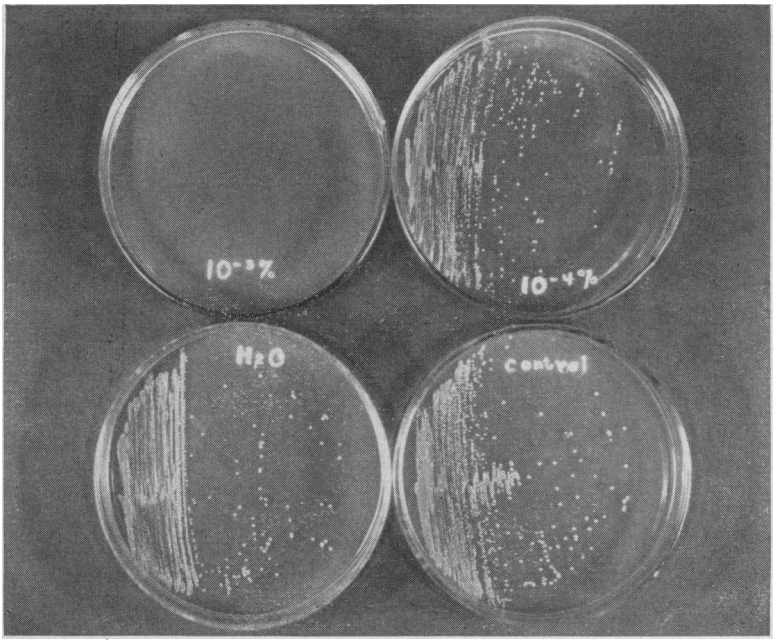

Figure 1. Effect of ether extract of hot-water filtrate of cotton in $10^{-3\%}$ and $10^{-4\%}$ concentrations compared with medium prepared with the extracted water.

using two volumes of ether for each extraction. The dried ether extracts were weighed, and titrated with N/10 NaOH, using phenolphthalein as an indicator. The mean molecular weight of the fatty acid extract was computed as 252.

Finally, the effect of adding to the basic medium the four principal fatty acids found in cottonseed oil was determined. Ethanol solutions of oleic, linoleic, palmitic, and stearic acids were added to the medium prior to autoclaving to give concentrations of 10, 1.0, and 0.1 mg. per liter. A control portion of the medium received the ethanol alone. The results obtained after 5 days of incubation are presented in table 2, and figure 2. Complete inhibition of growth was obtained with oleic and linoleic acids at concentrations of 10 and 1.0 mg. per liter and definite though incomplete inhibition with 0.1 mg. per liter. The saturated fatty acids gave incomplete but definite inhibition at concentrations of 10 and 1.0 mg. per liter. Inhibition by the above concentrations of fatty acids was not evident in the presence of 0.1 percent corn starch.

Discussion

In addition to demonstrating the role of fatty acids in inhibition resulting from filtration of media through cotton, the present studies

Figure 2. Effect of oleic, linoleic, palmitic, and stearic acids in concentration of 1 mg./liter, on *Br. abortus*, strain 2451.

Table 2. *Inhibition of* Br. abortus *No. 2451 by principal fatty acids of cottonseed oil*

Fatty acid	Mg/Liter		
	10	1.0	0.1
Oleic	0	0	1
Linoleic	0	0	1
Palmitic	1	1. 5	3
Stearic	1	2	3. 5

Incubation time—5 days.
Control—excellent (4) growth.

add *Br. abortus* to the growing list of organisms that may be susceptible to fatty-acid inhibition.

The widespread use of cotton for clarifying media, the possibility of transferring inhibitory lipids from cotton plugs to glassware during sterilization with dry heat, as shown by Wright (*3*) and by Drea (*4*), and the presence in agar, according to Gould and Mueller (*5*), and in peptone of substances whose inhibitory properties may be neutralized by starch, indicate the advisability of adding starch to media used for the isolation of fastidious organisms. Such a procedure would

appear to be preferable to complete removal of fatty acids from media in view of the lipid requirements of certain organisms as shown by Dubos and Davis (*6*) and by Pollock (*7*).

Summary and Conclusions

Filtration of an improved tryptose agar through 15 different commercial brands of absorbent cotton resulted in media which completely inhibited the growth of a strain of *Br. abortus*. The inhibition was due to substances extractable from cotton, which were soluble in hot water and soluble in ether after acidification with hydrochloric acid to pH 2.0. These fatty-acid-like substances completely inhibited growth of the test organism when added to the medium in the concentration of 10 mg. per liter. The unsaturated fatty acids which are found in cottonseed oil inhibited growth of the *Brucella* culture in a concentration of 1 mg. per liter; the saturated fatty acids gave partial inhibition at this concentration. The inhibition of growth could be neutralized by the addition of starch to the medium.

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Training Course in Rat-Borne Disease

The twelfth semiannual field training course in rat-borne disease prevention and control will be held by the Communicable Disease Center, Public Health Service, in Atlanta, March 12–30, 1951.

The course is designed for rat control specialists including personnel of large city and county health departments, State health departments, and the Public Health Service. Military personnel are welcome to attend these courses.

"Rat-Borne Disease Prevention and Control," a new Communicable Disease Center manual, is the basic handbook for the course. The more complex considerations in this manual will be visualized for the first time by the new joint Army-Public Health Service series of motion pictures on rat control.

There will be increased emphasis on: (1) Surveys of towns for rodent infestation and recommendations for a suitable control program; (2) organization of local programs; (3) individual contacts in the promotion of anti-rat sanitation programs; (4) training of subordinate personnel; and (5) development of improved presentation by technical personnel of programs to lay audiences.

Following the 3-week training period in rat-borne disease, the Center will give a 2-week concentrated field training course in the control of flies, mosquitoes, and other insect vectors of disease. Personnel interested in both rat and insect control may attend both courses. The dates for this course are April 2–13, 1951.

Environmental sanitation is stressed as one of the most important methods of both rat and insect control.

Applications for the courses may be sent to the Medical Officer in Charge, Communicable Disease Center, 50 Seventh Street NE., Atlanta, Ga., Attention: Chief, Training Services.

Incidence of Disease

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

Reports from States for Week Ended December 23, 1950

Measles. The number of cases of measles for the current week was 2,827 as compared with 2,008 for the same week last year. The total number reported since the seasonal low week is 24,588 as compared with 16,352 for the same period last year, and 45,079 in 1948. The only geographic divisions in which measles is now being reported in significantly greater numbers than for the 5-year (1945–49) median are the East North Central, West North Central, and West South Central States.

Other diseases. Influenza continues to be reported most frequently in a few States, namely in Texas (2,029 cases for the current week), Virginia (290), Arizona (214), and Arkansas (171). Ninety-five cases were reported in Hawaii where laboratory examination showed antibody rise for type A influenza virus in September and October. A 32-percent decrease in number of poliomyelitis cases from the previous week (368) was reported for the current week (251). The total

Comparative Data For Cases of Specified Reportable Diseases: United States

[Numbers after diseases are International List numbers, 1948 revision]

Disease	Total for week ended—		5-year median 1945–49	Seasonal low week	Cumulative total since seasonal low week		5-year median 1944–45 through 1948–49	Cumulative total for calendar year—		5-year median 1945–49
	Dec. 23, 1950	Dec. 24, 1949			1949–50	1948–49		1950	1949	
Anthrax (062)	1	1	(1)	(1)	(1)	(1)	(1)	45	52	(1)
Diphtheria (055)	94	118	290	27th	2,794	4,100	5,970	5,922	7,868	12,267
Acute infectious encephalitis (082)	14	12	7	(1)	(1)	(1)	(1)	994	750	615
Influenza (480–483)	3,161	2,289	3,338	30th	34,775	27,910	32,861	281,034	103,777	220,512
Measles (085)	2,827	2,008	2,696	35th	24,588	16,352	23,401	312,759	604,870	596,866
Meningococcal meningitis (057.0)	61	57	55	37th	883	844	844	3,682	3,360	3,362
Pneumonia (490–493)	1,085	1,415	-------	(1)	(1)	(1)	(1)	[2] 78,629	75,987	-------
Acute poliomyelitis (080)	251	154	138	11th	31,961	41,258	24,631	33,092	42,171	25,098
Rocky Mountain spotted fever (104)	4	-------	1	(1)	(1)	(1)	(1)	458	560	560
Scarlet fever (050)	1,215	1,083	1,697	32d	14,202	15,225	20,631	54,372	72,891	81,632
Smallpox (084)	-------	-------	4	35th	8	7	21	34	48	168
Tularemia (059)	24	32	32	(1)	(1)	(1)	(1)	878	1,106	1,106
Typhoid and paratyphoid fever (040, 041) [3]	43	59	41	11th	2,887	3,344	3,364	3,396	3,832	3,849
Whooping cough (056)	1,384	1,394	1,530	39th	[4] 20,215	20,066	22,690	[4] 117,410	66,668	98,565

[1] Not computed.
[2] Addition: Tennessee, week ended Dec. 16, 32 cases.
[3] Including cases reported as salmonellosis.
[4] Addition: Arizona, week ended Dec. 16, 44 cases.

number for 51 weeks of 1950 is now 33,092 as compared with 42,171 for the same period last year.

Reports of Epidemics

Infectious hepatitis. Dr. Kotcher, Kentucky State Department of Health, has reported two outbreaks of infectious hepatitis occurring in two widely separated areas of the State in one week. Only children were affected in each instance. One outbreak occurred in a single school where mothers helped with preparing the school lunch. One such mother had two children with the disease. The symptoms consisted of general malaise, abdominal pain, nausea and vomiting, constipation, and typical increasing jaundice.

Rabies in ferrets. Dr. W. L. Bierring, Iowa State Commissioner of Health, has reported an episode in which ferrets were shown to have rabies. A man with a number of ferrets appeared in a town in southern Iowa. He solicited business establishments to contract for elimination of rats, and sold animals to two persons. One ferret died 3 days after it was sold, and a second died 12 days after purchase. Rabies was proved in the latter animal by the Iowa State Veterinary Diagnostic Laboratory. The purchaser of the second ferret had been bitten by the animal and was given antirabies vaccine. The original owner of the ferrets was eventually located in Kansas through cooperation of various State agencies in Iowa and Kansas. The State Health Department of Kansas learned that the man had returned to his home in Kansas because his remaining ferrets had died of a disease which he thought was distemper. He, too, had been bitten and antirabies vaccine was advised.

(NOTE: In Iowa, 360 rabid animals have been reported this year, and Kansas has reported 48.)

Deaths During Week Ended December 23, 1950

	Week ended Dec. 23, 1950	*Corresponding week, 1949*
Data for 93 large cities of the United States:		
Total deaths	9, 470	9, 399
Median for 3 prior years	8, 890	----------
Total deaths, first 51 weeks of year	466, 949	467, 090
Deaths under 1 year of age	594	639
Median for 3 prior years	639	----------
Deaths under 1 year of age, first 51 weeks of year	31, 858	33, 231
Data from industrial insurance companies:		
Policies in force	69, 600, 434	69, 928, 911
Number of death claims	12, 173	12, 277
Death claims per 1,000 policies in force, annual rate	9. 1	9. 2
Death claims per 1,000 policies, first 51 weeks of year, annual rate	9. 2	9. 1

Reported Cases of Selected Communicable Diseases: United States, Week Ended December 23, 1950

[Numbers under diseases are International List numbers, 1948 revision]

Area	Diphtheria (055)	Encephalitis, infectious (082)	Influenza (480–483)	Measles (085)	Meningitis, meningococcal (057.0)	Pneumonia (490–493)	Poliomyelitis (080)
United States	**94**	*** 14**	**3,161**	**2,827**	**61**	**1,085**	**251**
New England	**1**		**5**	**163**	**4**	**35**	**6**
Maine			3	4	1	4	3
New Hampshire			2	28			
Vermont				41			
Massachusetts	1			41	3		1
Rhode Island				4		2	
Connecticut				45		29	2
Middle Atlantic	**8**	**1**	**5**	**532**	**14**	**278**	**43**
New York	7	1	[1] 2	158	5	170	33
New Jersey			3	31	1	66	3
Pennsylvania	1			343	8	42	7
East North Central	**8**	**4**	**29**	**800**	**6**	**107**	**49**
Ohio	5			168	1		14
Indiana			12		1	14	2
Illinois	1	2	1	111	3	46	10
Michigan	2	2		134	1	33	21
Wisconsin			16	387		14	2
West North Central	**13**	**2**	**18**	**332**	**7**	**68**	**20**
Minnesota	5			101		10	2
Iowa	2			1	1	1	7
Missouri	4	1	1	115	5	15	1
North Dakota			13	4	1	32	2
South Dakota		1		9			
Nebraska	2						4
Kansas			4	102		10	4
South Atlantic	**19**		**479**	**167**	**8**	**130**	**33**
Delaware				1			
Maryland	1			4	2	22	3
District of Columbia				8	1	10	
Virginia	2		290	22		47	4
West Virginia			85	7		10	1
North Carolina	11			79	4		6
South Carolina	3		48	2	1	21	1
Georgia	1		52	36		12	11
Florida	1		4	8		8	7
East South Central	**15**	**1**	**54**	**26**	**4**	**40**	**8**
Kentucky	4			2	2	11	1
Tennessee	8		24	12	1		1
Alabama	2		29	3	1	24	4
Mississippi	1	1	1	9		5	2
West South Central	**20**	**2**	**2,294**	**311**	**12**	**348**	**26**
Arkansas	3	1	171	73	1	27	3
Louisiana	3		1	7	1	30	8
Oklahoma	3		93	21	1	19	5
Texas	11	1	2,029	210	9	272	10
Mountain	**2**	**1**	**273**	**249**	**2**	**52**	**11**
Montana	2		8	7		1	1
Idaho			40	94		8	
Wyoming		1		4		1	1
Colorado			10	97	2	10	2
New Mexico				22		2	3
Arizona			214	12		29	2
Utah			1	11		1	2
Nevada				2			
Pacific	**8**	**3**	**4**	**247**	**4**	**27**	**55**
Washington				111			13
Oregon	4		1	16		8	9
California	4	3	3	120	4	19	33
Alaska						4	4
Hawaii			95				

[1] New York City only.

Anthrax: Pennsylvania, 1 case.

Reported Cases of Selected Communicable Diseases: United States, Week Ended December 23, 1950—Continued

[Numbers under diseases are International List numbers, 1948 revision]

Area	Rocky Mountain spotted fever (104)	Scarlet fever (050)	Smallpox (084)	Tularemia (059)	Typhoid and paratyphoid fever [1] (040,041)	Whooping cough (056)	Rabies in animals
United States	**4**	**1,215**		**24**	**43**	**1,384**	**83**
New England		**106**			**1**	**212**	
Maine		11				52	
New Hampshire		2				3	
Vermont		4				43	
Massachusetts		67			1	79	
Rhode Island		7				17	
Connecticut		15				18	
Middle Atlantic	**1**	**185**			**8**	**247**	**12**
New York	1	[2] 124			1	102	12
New Jersey		17				75	
Pennsylvania		44			7	70	
East North Central		**272**		**6**	**3**	**250**	**13**
Ohio		42		1		40	
Indiana		23				9	8
Illinois		46		4		37	
Michigan		119			1	84	5
Wisconsin		42		1	2	80	
West North Central		**77**			**7**	**72**	**8**
Minnesota		11				19	1
Iowa		10			1	1	7
Missouri		25			4	8	
North Dakota		2				23	
South Dakota		1					
Nebraska		2				4	
Kansas		26			2	17	
South Atlantic	**1**	**143**		**5**	**8**	**196**	**13**
Delaware		7				8	
Maryland		7		2		17	
District of Columbia		8			2	3	
Virginia	1	20		1	1	48	1
West Virginia		7				9	2
North Carolina		69				81	
South Carolina		3			1	1	4
Georgia		12		2	4	16	6
Florida		10				13	
East South Central	**2**	**79**		**5**	**5**	**33**	**9**
Kentucky		11				10	5
Tennessee		50		2	2	14	
Alabama	1	15			3	7	4
Mississippi	1	3		3		2	
West South Central		**76**		**7**	**5**	**211**	**28**
Arkansas		12		2	3	16	3
Louisiana		7		3		3	
Oklahoma		11				14	1
Texas		46		2	2	178	24
Mountain		**88**		**1**	**1**	**89**	
Montana		5		1		19	
Idaho		30				1	
Wyoming						2	
Colorado		17				10	
New Mexico		3				11	
Arizona		10			1	45	
Utah		23				1	
Nevada							
Pacific		**189**			**5**	**74**	
Washington		56				29	
Oregon		19			1	7	
California		114			4	38	
Alaska						2	
Hawaii		3					

[1] Including cases reported as salmonellosis.
[2] Including cases reported as streptococcal sore throat.

FOREIGN REPORTS

PANAMA

Poliomyelitis. During the period September 1 to December 7, 1950, 68 cases (3 deaths) of poliomyelitis were reported in Panama, of which 26 cases were in the Canal Zone. For the 3-month periods September–November in 1945 to 1948 the maximum number was five cases. Data for 1949 are not available. There were 18 cases reported in Panama City during the period considered in 1950.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently. A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

Pakistan. During the week ended November 18, 1950, three fatal cases of cholera were reported in Dacca.

Smallpox

Cambodia. During the week ended December 9, 1950, six cases of smallpox were reported in Takeo Province.

Gambia. For the week ended November 11, 1950, one case of smallpox was reported in Gambia.

Indonesia. During the week ended November 25, 1950, 18 cases of smallpox were reported in Surabaya, Java, and 50 cases were reported in Bandjermasin, Borneo. For the week ended November 4, 1950, 17 cases of smallpox were reported in Pontianak, Borneo, as compared with 4 for the previous week.

Pakistan. During the weeks ended December 9 and 16, 1950, three cases of smallpox were reported each week in Karachi.

Typhus Fever

India. For the week ended December 9, 1950, four cases of typhus fever were reported in Bombay as compared with three for the week ended December 2.

Turkey. During the week ended December 16, 1950, 11 cases of typhus fever were reported in Turkey, 1 of which was reported in Istanbul.

Gold Coast. The suspected case of yellow fever reported in Accra on October 11, 1950 and the suspected fatal case reported in Taquah-Aboso have both been confirmed. (See PUBLIC HEALTH REPORTS, November 17, 1950, p. 1535 and December 8, 1950, p. 1655, respectively.)