# Public Health Reports

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## AN EPIDEMIC OF A SEVERE PNEUMONITIS IN THE BAYOU REGION OF LOUISIANA [1]

### III. PATHOLOGICAL OBSERVATIONS. REPORT OF AUTOPSY ON TWO CASES WITH A BRIEF COMPARATIVE NOTE ON PSITTACOSIS AND Q FEVER

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An epidemic of unusually severe pneumonitis with a high mortality rate occurred in southwest Louisiana during the interval December 1942 to May 1943. This is a report of the post-mortem pathology in two cases of the disease, the clinical features of which have been described in the preceding article (*1*). The findings are compared with those seen in psittacosis and Q fever.

*Case 17.*—Miss B., age 39, who died on the 10th day of illness (patient of Dr. E. L. Landry).

The autopsy was begun 5½ hours after death. The body had been kept in a very warm room at the funeral home where the examination was performed. No facilities for weighing organs were available.

External examination revealed an obese white woman. The only skin change noted was a vesicle about 1½ cm. in diameter on the lateral surface of the right thigh.

#### GROSS EXAMINATION

*Abdomen.*—The peritoneum was smooth and glistening. The liver presented about 5 cm. below the costal margin.

*Heart.*—The pericardial fluid was clear and the amount slightly increased. The myocardium was firm and of reddish-brown color. The epicardium, endocardium, and valves appeared normal.

*Lungs.*—The left pleural cavity contained several hundred cubic centimeters of clear fluid in which a few fibrin threads were seen. The right pleural cavity was dry. There were a number of delicate adhesions uniting the visceral and parietal layers of the pleura.

Left lung: The entire lower lobe and three-fourths of the upper lobe were consolidated. The consolidated areas presented a unique deep purplish plum color.[3]

[1] Presented to the Louisiana Association of Pathologists, December 30, 1943.

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[3] The nearest readily available match to this color is that represented on plate 47: J 9, 10, and L 9, 10, p. 117. *in* A Dictionary of Color—Maerz, A. J., Paul, M. R. New York, McGraw-Hill, 1930.

In the upper lobe there were multiple areas of complete consolidation alternating with and sharply demarcated from entirely normal lung tissue. On section similar sharp demarcation was evident and consolidated areas usually reached the pleural surface. The pattern of this consolidation was entirely different from that usually seen in bronchopneumonia or lobar pneumonia.

The consolidated lung was firm and noncrepitant to palpation, and somewhat less firm and more resilient than liver on section. The cut surface was very dry, the color dark purplish plum, and the bronchi empty.

Right lung: The lower lobe was almost completely replaced by a consolidation similar to that seen in the left lung, but its consistency was not as firm, and little crepitation could be elicited. The middle and upper lobes presented relatively small areas of the same type of consolidation. Again the bronchi were empty.

*Tracheobronchial lymph nodes.*—There was very little enlargement.

*Liver.*—There appeared to be slight enlargement. The anterior surface of the right lobe exhibited poorly defined yellowish mottling which extended 2 to 5 mm. into the parenchyma.

*Gall bladder.*—The organ contained 6 faceted cholesterol stones.

*Gastrointestinal tract.*—The stomach contained a small amount of reddish-black fluid. In the cardiac mucosa were two small depressions which appeared to be ulcers of recent origin. No lesions were found in the duodenum. On external examination the small and large intestine appeared normal.

*Spleen.*—The surface was smooth, the size about twice normal, and the deep red pulp so very soft that it was considerably torn in removing the organ from the body. Follicles were not seen.

*Uterus.*—Of normal size. On the serosal surface there were several firm, white tumors ranging up to 1 cm. in diameter.

*Pancreas, kidneys, suprarenal glands, urinary bladder, ovaries, and tubes.*—These organs appeared normal.

*Cranial cavity.*—Very little cerebrospinal fluid was noted. The dura appeared normal. There was an area of subarachnoid hemorrhage 2 or 3 cm. in diameter adjacent to the sagittal suture at the level of the right central sulcus. The leptomeninges appeared normal. The blood vessels of the white matter were prominent. The pituitary gland was of normal size and appearance.

## MICROSCOPIC EXAMINATION

Stains used were buffered Romanowsky (*2*) and van Gieson-Weigert iron hematoxylin.

*Heart.*—Sections of the left ventricle and of the interventricular septum showed a few clusters of mononuclear cells in the epicardium and distributed around the interstitial capillaries and larger vessels within the myocardium. There was a mild degree of fiber hypertrophy.

*Aorta.*—No lesions were seen. An abdominal periaortic lymph node revealed widening of the sinuses in which large macrophages were observed.

*Lung.*—Many blocks were studied. There were no significant changes in the pleura.

In the consolidated areas the alveoli were rather uniformly filled by an exudate which varied somewhat in composition from block to block. Most commonly it consisted of many large mononuclear cells, many necrobiotic cells with rounded pyknotic nuclei, some large phagocytic cells with ingested nuclear fragments or an occasional red corpuscle, a few lymphocytes, and many erythrocytes in a matrix of serofibrin. Neutrophils were generally sparse, often absent, and fairly numerous in only occasional microscopic fields. The large mononuclear cells were generally about 15 micra in diameter and exhibited fairly large, leptochromatic, rounded,

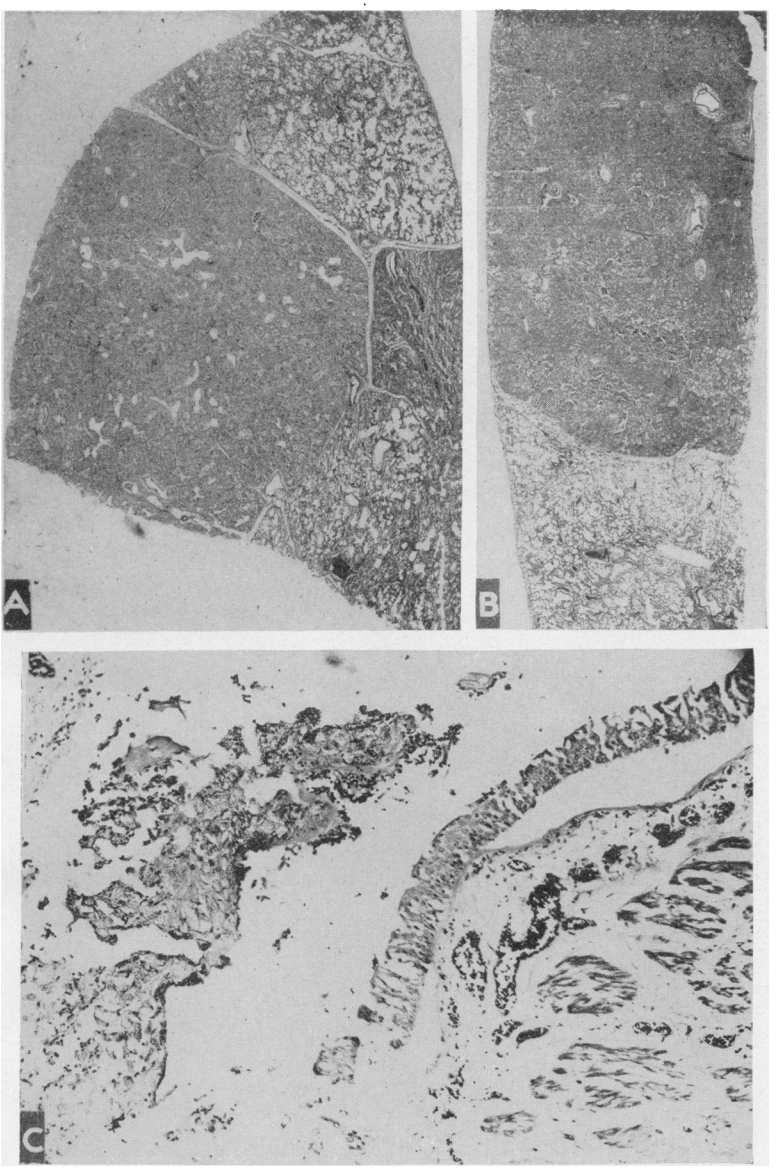


FIGURE 1.—Case 17. A and B. Lung exhibiting sharply defined areas of consolidation; van Gieson stain ×3.5. C Large bronchus showing stringy mucoid exudate and lack of inflammation in wall; Romanowsky stain ×90.

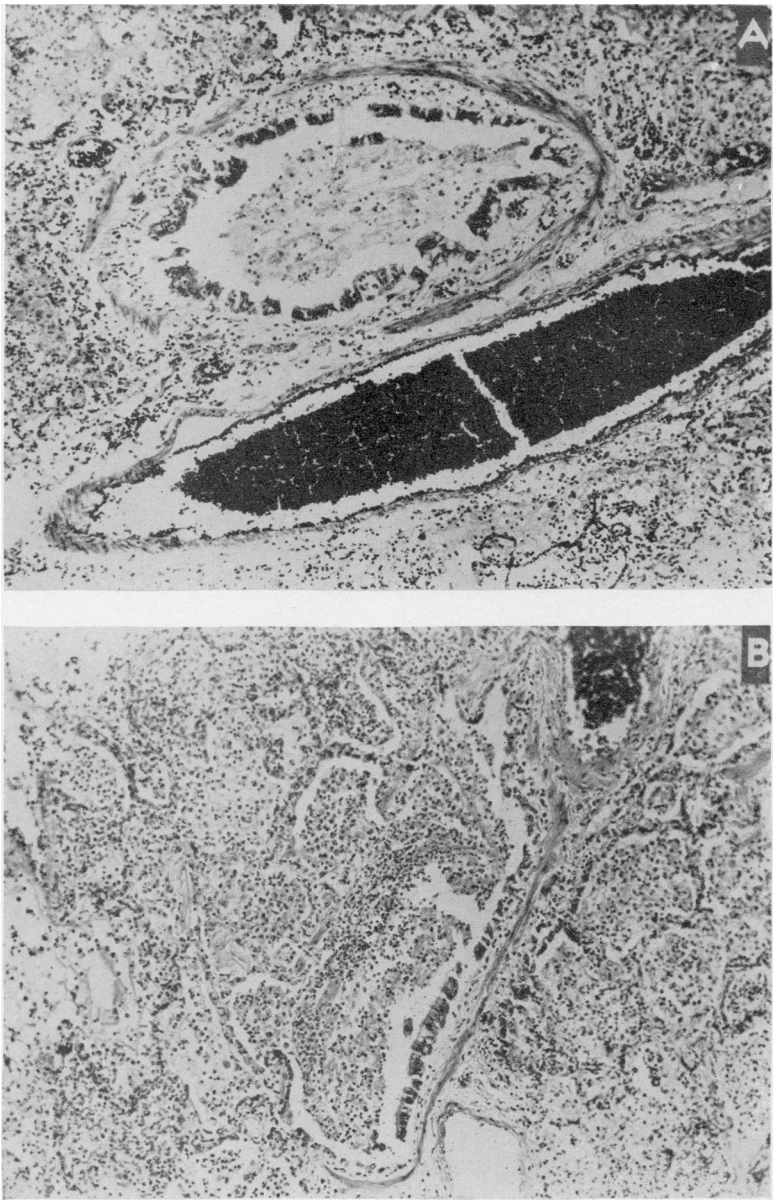


FIGURE 2.—Case 17. A. Bronchiole and blood vessel in area of pneumonia. Thin mucocellular exudate in bronchiole and normal wall; Romanowsky stain ×90. B. Respiratory bronchiole showing extension of exudate from alveolar side while bronchiolar mucosa is intact; Romanowsky stain ×90.

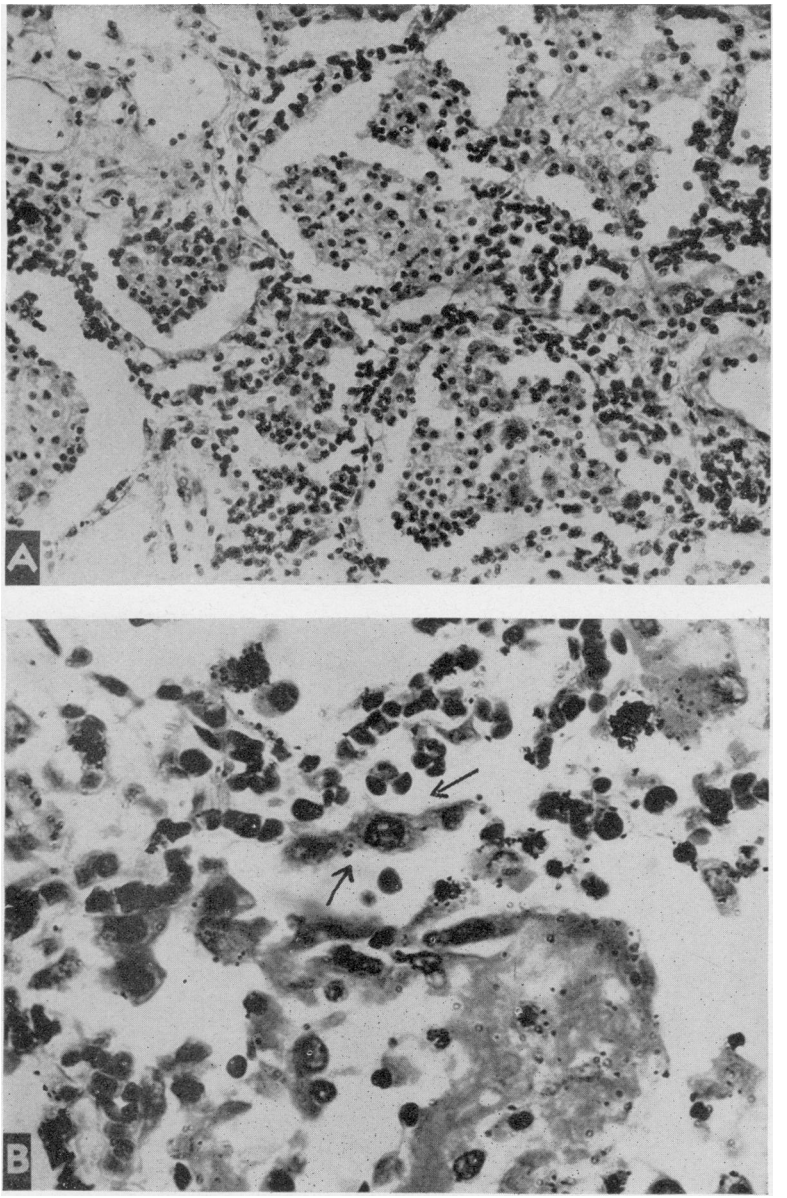


FIGURE 3.—Case 17. A. Lung showing alveolar exudate of large mononuclear cells. Romanowsky stain ×250. B. Swollen alveolar epithelial cells and fibrinous exudate. Romanowsky stain ×470.

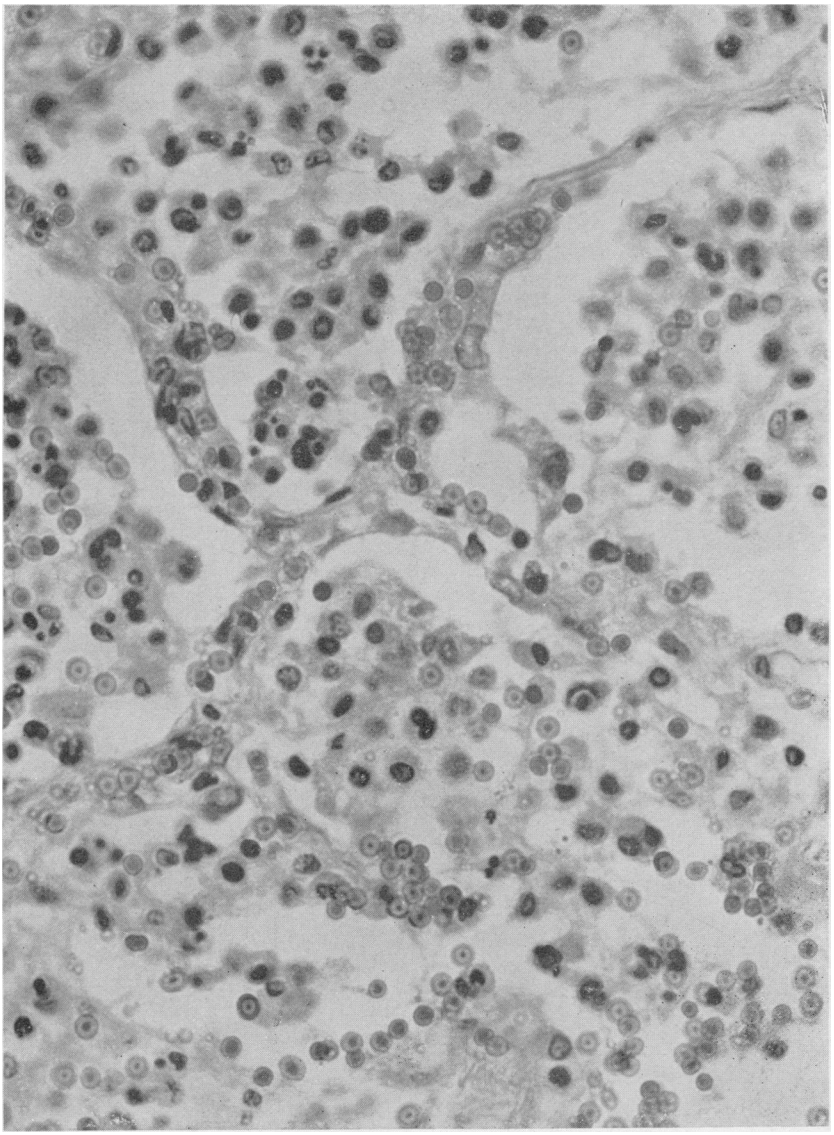

FIGURE 4.—Case 17. Large mononuclear cell exudate in pneumonic lung; Romanowsky stain ×700.

indented, or lobate nuclei, and wide amphophil cytoplasmic zones. A few cells in the alveolar exudate were seen in mitosis.

Generally, in the consolidated areas the alveolar lining cells were conspicuous, very large, hyperchromatic, and their cytoplasm widened, finely vacuolated, and often basophilic. Occasional mitotic figures were observed. Many desquamated lining cells appeared in the exudate. In areas where this hyperplasia was most marked, the alveolar exudate contained much fibrin and relatively few mononuclear cells. The alveolar septa were uniformly congested but showed only small areas of mononuclear cell infiltration. In several microscopic fields the septal outlines were lost.

The respiratory bronchioles often exhibited the characteristic exudate extending into their lumina from the alveolar side, while their bronchial epithelium remained intact. A seromucinous exudate with few leucocytes filled the terminal bronchioles. Even in densely consolidated zones their walls and mucosa revealed only slight involvement. The epithelium was intact and in areas showed a few emigrating neutrophils. The walls were generally only moderately edematous and slightly or moderately infiltrated by mononuclear cells. The large bronchioles and bronchi exhibited still less alterations of the same general type.

Mild mononuclear cell infiltration was seen around some of the smallest arterioles and venules. The larger arterioles and the arteries showed no significant changes. Thrombi were seen in a few small arteries and occasionally in capillaries.

Several sections included the sharp zones of demarcation between consolidated and nonconsolidated lung. Often this line followed the course of an arteriole or venule and in their absence a thin connective tissue septum, but in some areas no dividing structure was seen. The septal capillaries in the air-containing areas were moderately congested. In sections from areas without obvious consolidation the septal capillaries were engorged and alveoli partly filled with a serosanguinous exudate.

*Primary bronchus.*—The mucosa, submucosa, and wall showed no significant changes.

*Tracheobronchial lymph nodes.*—There was widening of the peripheral and some of the pulp sinuses. The latter were filled with mononuclear cells. Follicles were hypoplastic.

*Liver.*—The sinusoids were congested, especially in the centrolobular zones. The Kupffer cells showed considerable swelling and much phagocytosis of lymphocytes and erythrocytes. The sinusoids contained mononuclear cells, some of which had ingested erythrocytes, and serum. Rarely a few of the periportal parenchymal cells were finely vacuolated.

*Spleen.*—The follicles were very small. The entire pulp showed intense infiltration by lymphocytes, plasma cells, and large phagocytic cells with pale, oval nuclei and foamy cytoplasm. Erythrocytes were mixed with this infiltrate in considerable numbers. Much blood pigment was present. Neutrophils were few and played no conspicuous part in this reaction. The sinus architecture was practically obliterated by this intense infiltration; but where perceptible, the lining reticulo-endothelial cells were swollen.

*Stomach.*—A section taken near the cardiac sphincter showed a small ulcer with overhanging edges. The base exhibited a few coagulated, vaguely perceptible, mucosal glands and capillaries filled with tiny thrombi. Other parts of the gastric mucosa showed no changes.

*Kidney.*—The glomerular capillaries were congested. Some capsules contained little granular acidophilic exudate. The convoluted tubules were lined by swollen epithelial cells with acidophilic granular cytoplasm. These tubules frequently contained a little albuminous exudate.

*Rectus muscle.*—Striations were lost in most of the fibers and partly or completely lost in others. Sarcoplasm was swollen, opaque, and eosinophilic, and interstitial nuclei prominently increased.

*Bone marrow, vertebral.*—The marrow was cellular. No changes were noted.

*Brain.*—Numerous sections of cerebellum, cerebral cortex, corpus striatum, thalamus, pons, and medulla were studied. The hemorrhagic areas described in the gross protocol was limited to the leptomeninges and appeared to be of very recent origin. The white matter of the cerebral cortex and brain stem revealed minute perivascular areas of hemorrhage and a few small sharply circumscribed zones of demyelinization in which the ground substance stained poorly and was finely vacuolated. The dura and choroid plexus showed no lesions. The pituitary appeared normal.

Sections of pancreas, small intestines, colon, suprarenal, pituitary, urinary bladder, ovary, and uterus appeared normal, with the exception of the leiomyomata in the last named.

*Bacteriology.*—At the autopsy table cultures were made of lungs, heart blood, liver, spleen meninges, and brain on blood agar and in thioglycollate broth. No pathogenic bacteria were recovered. Smears made of the same organs revealed no bacteria. Sections of pneumonic lung and spleen were stained for bacteria by Gram's method, toluidine blue, Giemsa's method, and Steiner's silver method. No bacteria were identified.

Pneumonic lung sections stained by the Romanowsky method were studied carefully using a 2-mm. oil immersion objective. After prolonged search several of the alveolar lining cells and a few free cells of a type with elongated leptochromatic nuclei presented in their cytoplasm clusters of delicate minute coccobacillary bodies which stained a deep purplish blue. Often the coccobacillary forms exhibited bipolar basophil granules and a faintly outlined pale blue connecting central part. In the liver a single Kupffer cell was found with similar bodies. Spleen sections showed none.

### SUMMARY OF CASE 17

A firm pneumonic consolidation characterized by a deep purplish plum color and sharp lines of demarcation separating it from nonpneumonic lung [3] almost completely replaced the left lung and the right lower lobe, while the middle and right upper lobes were partially involved. Microscopically a compact serosanguino-fibrino cellular exudate filled the alveoli. Large mononuclear cells predominated. Neutrophils were conspicuously few. Alveolar lining cells were hyperplastic. Septa were congested, and in areas very little widened. The exudate extended into the respiratory bronchioles but only slightly affected the other bronchioles, and the bronchi were normal.

Related changes in other organs were swelling of Kupffer cells and large mononuclear cell accumulation in liver sinusoids, acute splenitis, two small recent ulcers in cardiac portion of stomach, acute hyalin necrosis of rectus muscle, a small subarachnoid hemorrhage, and petechial hemorrhages and anoxic necrosis of brain.

Nonrelated abnormalities were cholelithiasis and small uterine leiomyomata.

No bacteria were demonstrated in smears or tissue sections. No pathogenic bacteria were obtained on culture.

In a few alveolar lining cells and in a few free cells in the alveolar exudate clusters of basophil, cytoplasmic, minute coccobacillary inclusions were found. Similar inclusions were seen in one Kupffer cell in the liver.

[3] It is probable that the anatomic basis for the peculiar, sharply demarcated pattern of consolidation seen in this case and in case 18 is the "secondary" pulmonary lobule which is described and illustrated by Miller in his monograph. (Miller, W. S.: The Lung. Charles C. Thomas Co., Baltimore, 1937.)

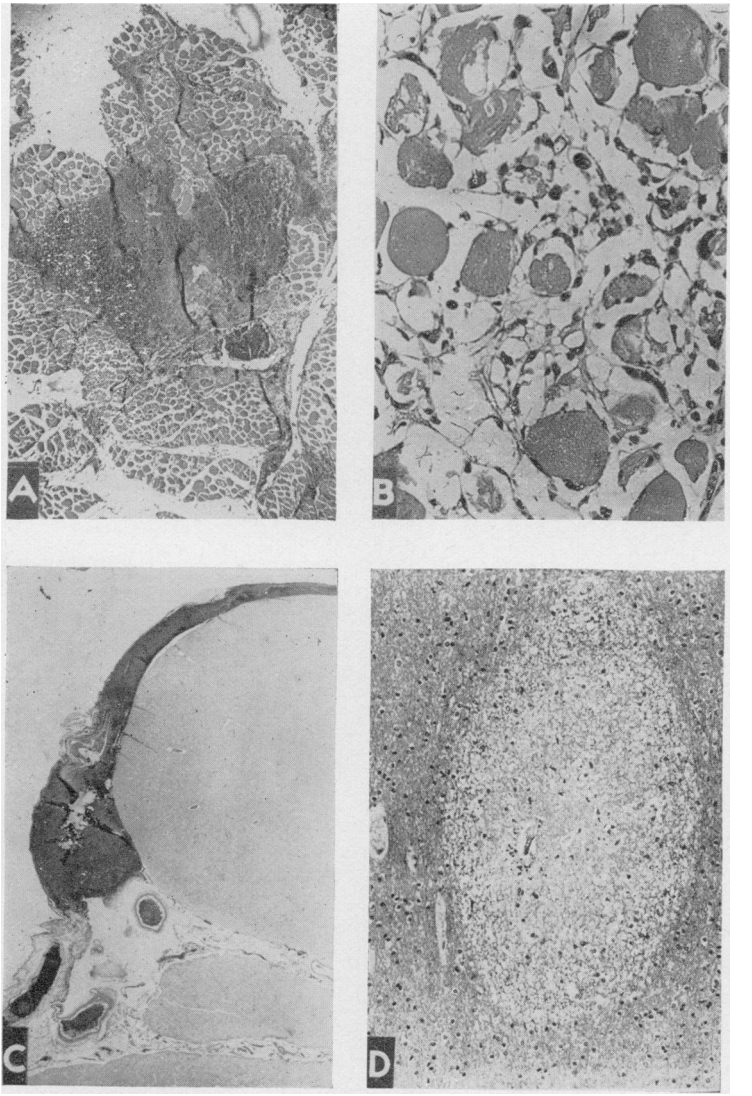


FIGURE 5.—A. Case 18. Hemorrhage in rectus muscle; van Gieson stain ×30. B. Case 18. Degeneration in rectus muscle; van Gieson stain ×150. C. Case 17. Area of subarachnoid hemorrhage; Romanowsky stain ×9.8. D. Case 17. Brain showing petechial hemorrhage and necrosis; van Gieson stain ×145.

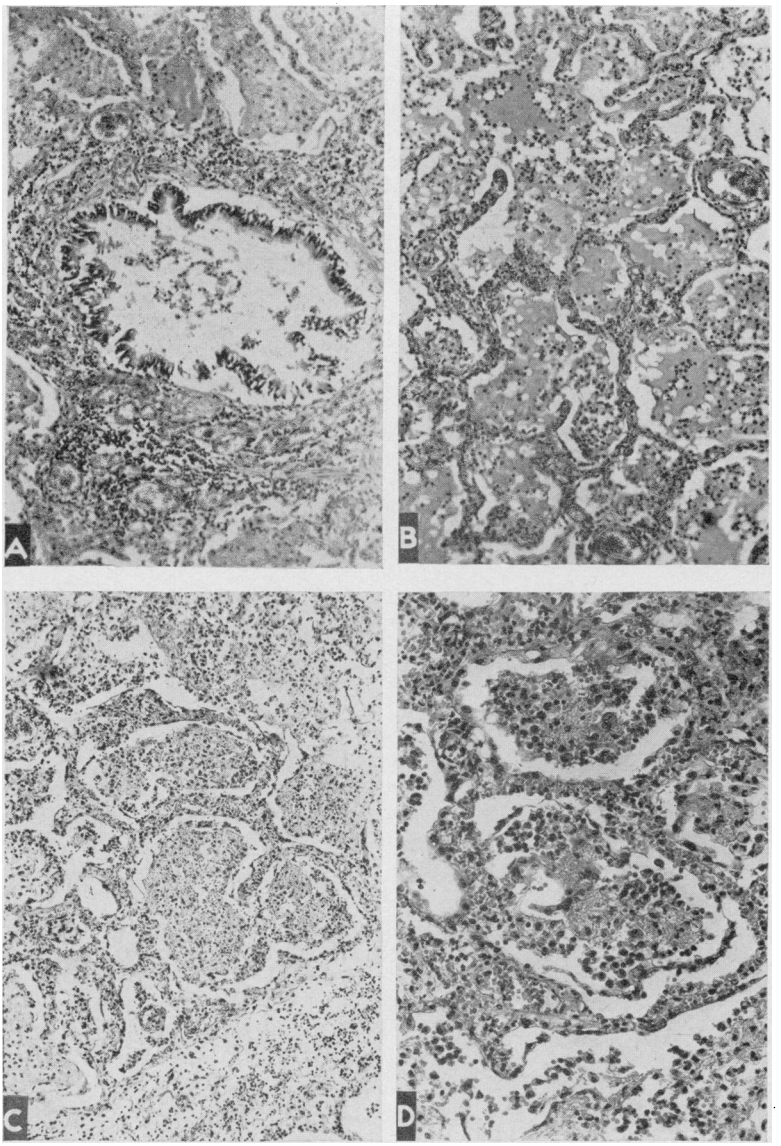


FIGURE 6.—Case 18. A. Relatively uninvolved bronchiole in pneumonic lung; van Gieson stain ×60. B. Serocellular exudate in alveoli; van Gieson stain ×60. C. Area of pneumonia with large mononuclear cell exudate; van Gieson stain ×60. D. Same as C ×120.

*Case 18.*—Miss B., age 41, who died on the 13th day of illness (patient of Dr. E. L. Landry). Autopsy was begun 10 hours after death and, with the exception that some refrigeration had been effected by placing ice about the body, the same conditions existed as in the previous case. External examination revealed a small white woman. No lesions of the skin and no jaundice were present.

GROSS EXAMINATION

*Abdomen.*—The right rectus muscle exhibited a hemorrhage approximately 10 by 5 by 1 cm. In the left rectus was a similar but smaller hemorrhage. The liver edge was about 5 cm. below the costal margin.

*Thorax.*—About 400 cc. of clear fluid were in the left pleural cavity and a slightly smaller amount in the right.

*Heart.*—There was a slightly increased amount of clear pericardial fluid. The right side of the heart was moderately dilated. The pericardium, myocardium, and valves were normal.

*Lungs.*—The four major lobes presented varying amounts of consolidation estimated as follows: Right lower, 90 percent; right upper, 75 percent; left upper, 75 percent; and left lower, 50 percent. The middle lobe was apparently uninvolved. The color of the consolidated areas was the same deep purplish plum which was mentioned before. The involved and uninvolved parenchyma were likewise delineated by clear-cut sharp lines of demarcation. The pleural surfaces were negative except for a very small area (1 cm.) of fibrinous exudate on the surface of the right lower lobe. The consistency of the consolidated areas was firm and again somewhat resembled that of liver but was more resilient.

The cut surfaces were wet, and considerable serosanguinous exudate was obtained on scraping. The color was uniformly the same deep purplish plum. The lines of demarcation between the involved and uninvolved portions followed the patterns above described. The bronchi contained small amounts of thin brownish fluid. There was very little enlargement of the tracheobronchial lymph nodes.

*Liver.*—The surface presented a diffuse, mild, subcapsular, hemorrhagic mottling. On section the cut surface had a smooth and homogeneous appearance. The gall bladder was normal.

*Spleen.*—The organ was about twice the normal size. The capsule was smooth. On section the consistency was firm and the cut surface was even with or slightly elevated above the capsule. The follicles were not easily distinguished. The pulp had a dark, reddish purple color.

*Kidneys.*—Estimated weight of each 175 gm. The capsules stripped easily, leaving a smooth, gray surface. The cortex and medulla were sharply demarcated; the medulla was congested. The cortex was pale gray and somewhat swollen. The pelves and ureters appeared normal.

*Gastrointestinal tract.*—No distinct lesions were noted in the stomach and duodenum. On inspection and palpation the remainder of the intestinal tract was normal. The appendix was absent.

*Pancreas.*—The size was normal. In the body a calculus 8 mm. in diameter was found occluding the pancreatic duct, but no necrosis was present. The suprarenal glands, abdominal periaortic lymph nodes, urinary bladder, ovaries, tubes, and thyroid were apparently normal. There were several fibroid tumors in the uterus.

*Cranial cavity—brain.*—The subarachnoid fluid appeared to be somewhat increased in amount. The brain was placed in 4 percent formaldehyde and dissected after hardening. No lesions were seen on multiple coronal sections. The pituitary was normal in size and appearance.

MICROSCOPIC EXAMINATION

The same stains were used as in case 17.

*Heart.*—In the epicardium there was mild lymphocyte and plasma cell infiltration. The myocardium of both ventricles presented interstitial edema and mild to moderately severe lymphocyte and plasma cell infiltration which was increased about the small blood vessels. There was a tendency for the infiltrating cells to be grouped about the small blood vessels.

*Aorta.*—No lesions.

*Lungs.*—Sections made through many of the consolidated areas presented in general a fairly uniform picture which varied little from that seen in the previous case. There was a fibrinocellular or serocellular exudate filling the alveoli. The cellular composition of the exudate was predominantly of a nonpolymorphonuclear-cell type. Erythrocytes were present in fairly large numbers. Large mononuclear cells again predominated. Characteristically they presented rounded, lobate, or kidney-shaped, excentrically placed, leptochromatic nuclei and broad amphophil cytoplasm. Some macrophages contained phagocytozed particles of nuclei and carbon. There was little erythrophagia. In many large round cells the nuclei were pyknotic and fragmented. Occasional cells in the exudate were undergoing mitotic division. Neutrophils, though generally rare, in a very few fields were moderately numerous. A few lymphocytes were present also.

The alveolar lining cells were large and conspicuous, their nuclei large, frequently rounded and deeply stained, and their elongated cytoplasm vacuolated. A few cells were undergoing mitotic division. Desquamation was common, and the cells formed a small part of the alveolar exudate. Though obliterated in a few small areas, the interalveolar septa generally were easily distinguished, moderately widened, and usually congested. They contained a medium number of mononuclear cells. In some fields acidophil thrombi were easily discernible in the lumina of the capillaries.

The respiratory bronchioles often showed an extension of the alveolar exudate into their lumina, while the bronchial mucosa remained intact and essentially unchanged. Terminal and larger bronchioles contained mucinous exudate with few leucocytes. Their walls were moderately edematous and only slightly infiltrated by lymphocytes and plasma cells and occasional neutrophils.

Small blood vessels were sometimes surrounded by lymphocytes. In several of the medium-sized arteries the lumina were partly filled by compact thrombi which usually were adherent to the intima. The walls of the larger arteries revealed no inflammatory reaction. In a few sections small, sharply defined areas revealed early coagulation necrosis of the septa and blood-filled alveoli.

In several of the lung blocks some air-containing parenchyma was present, and in one block, with the exception of a little serous exudate, it appeared to be entirely normal. The pleura exhibited no abnormality.

*Primary bronchus.*—No lesions.

*Tracheobronchial lymph node.*—Follicles were inactive. Dilated peripheral and pulp sinuses were filled with mononuclear cells.

*Liver.*—Immediately beneath the capsule were foci measuring up to 0.2 mm. in diameter which exhibited vacuolated oxyphilic coagulated liver cells and partly clotted blood in the dilated sinusoids. Deeper in the block but not over 5 mm. from the capsule an occasional centrolobular fibrino-mononuclear-cell thrombus was observed in the sinusoids. Throughout the sections there was enlargement, vacuolization, and marked phagocytic activity in Kupffer cells. In the sinusoids also were medium numbers of large, round phagocytic cells and large mononuclear cells. A few lymphocytes were also present, but neutrophils were only rarely noted.

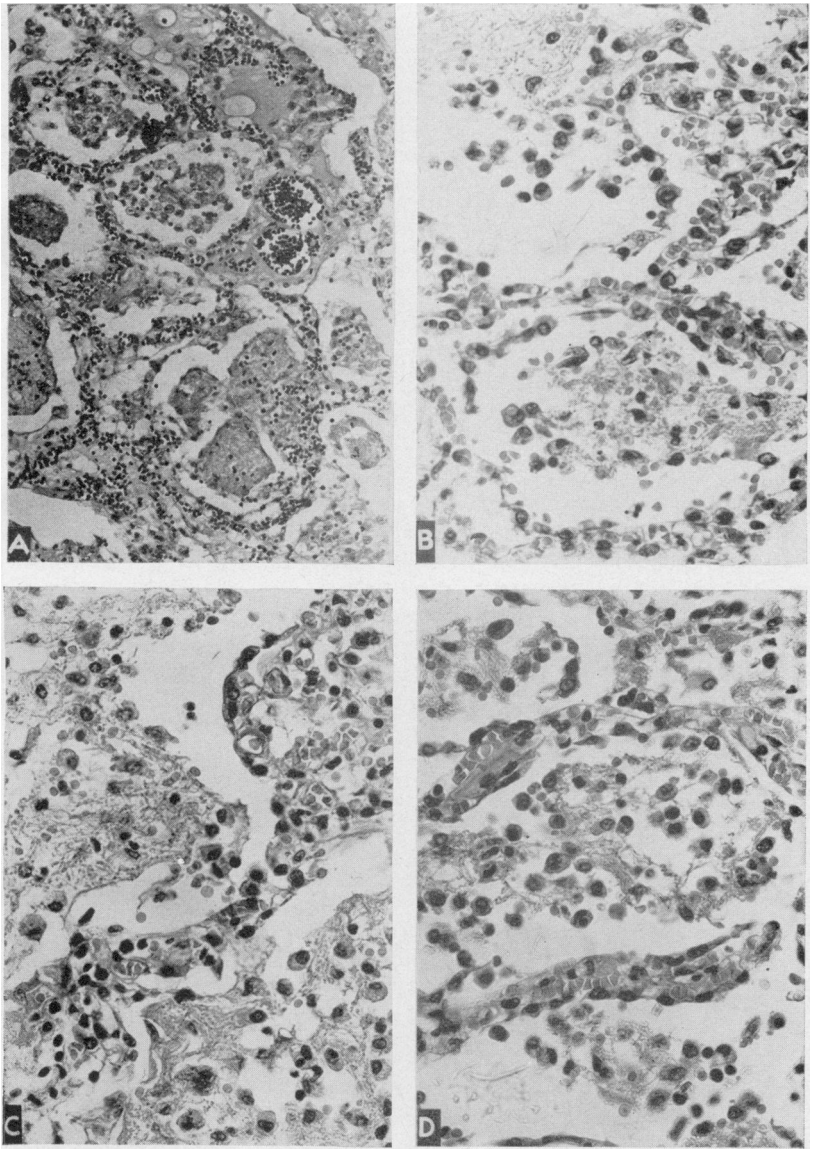


FIGURE 7.—Case 18. A. Lung with fibrinocellular exudate; Romanowsky stain ×120. B. Lung. Note swelling of alveolar lining cells; van Gieson stain ×350. C. Lung showing large mononuclear cell alveolar exudate and slightly widened septa; van Gieson stain ×350. D. Another area of pneumonia. Note swollen alveolar lining cells; van Gieson stain ×350.

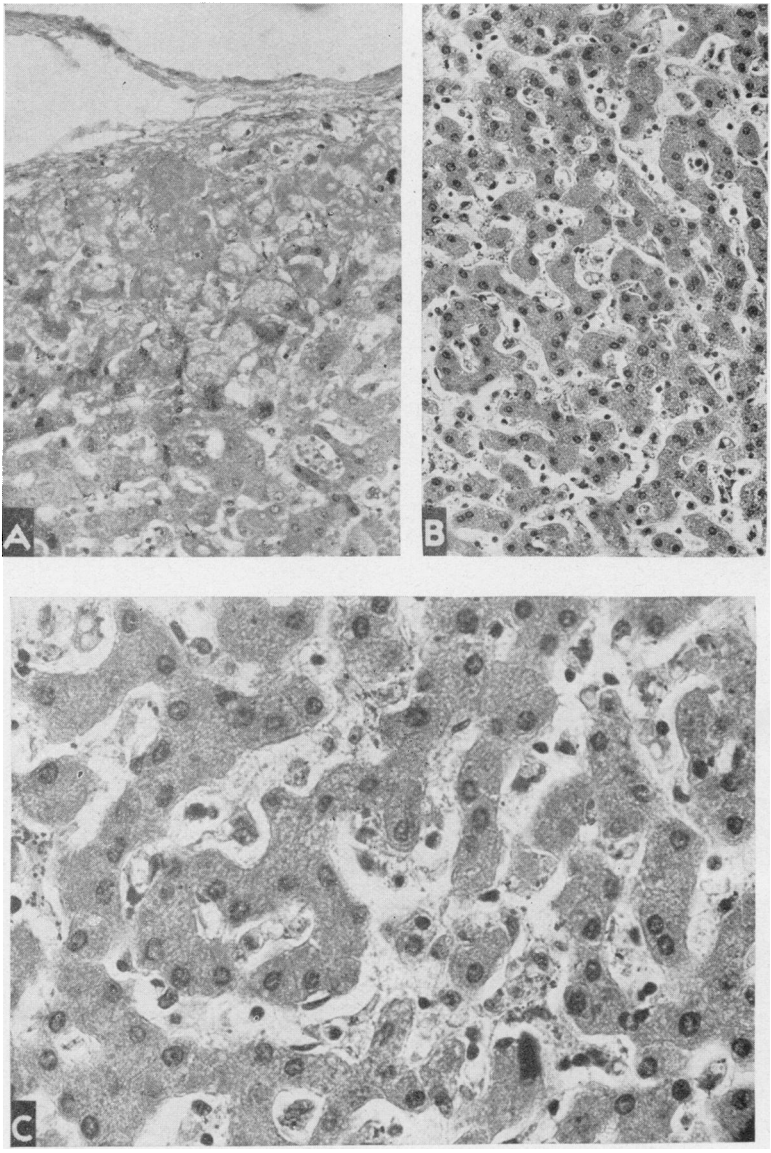


FIGURE 8.—Case 18. A. Liver. Subcapsular necrosis; Romanowsky stain ×160. B. Liver, non-necrotic part; van Gieson stain ×160. C. Liver; van Gieson stain ×450.

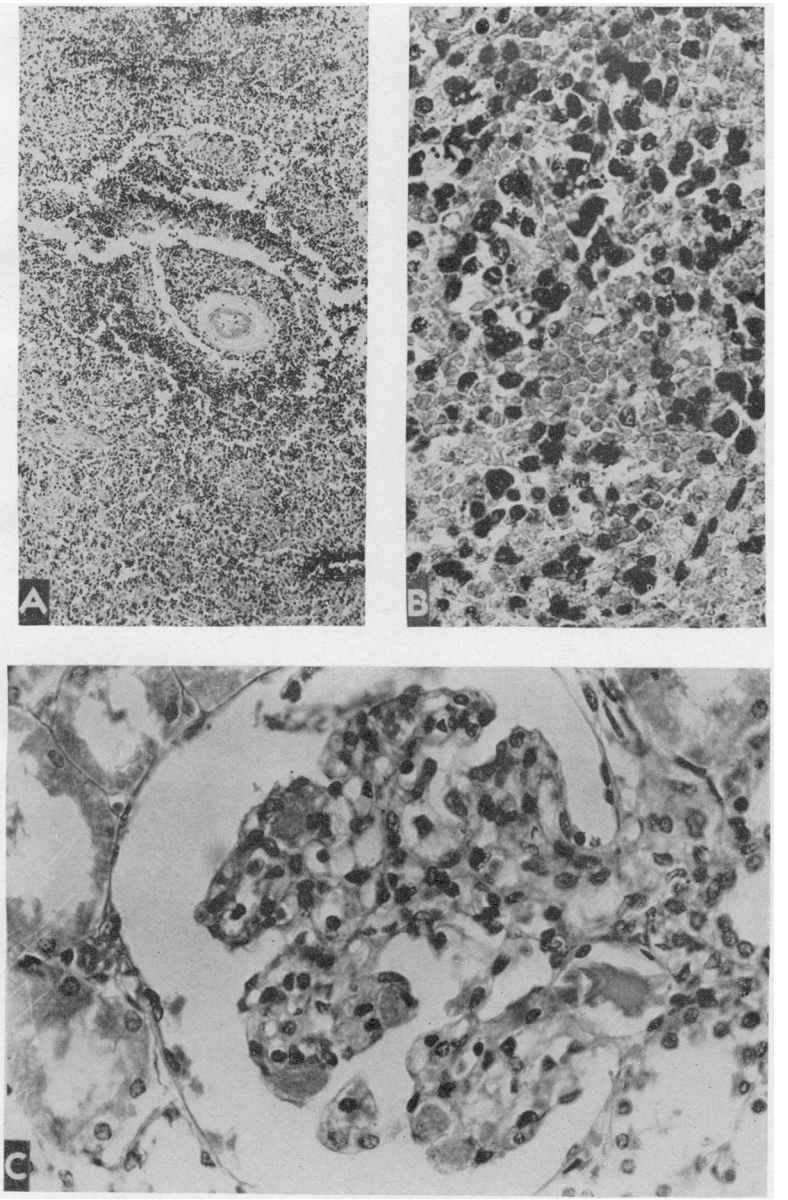


FIGURE 9.—A. Case 17. Spleen; Romanowsky stain ×100. B. Case 18. Spleen; van Gieson stain ×450. C. Case 18. Kidney; thrombi in glomerular capillaries; van Gieson stain ×450.

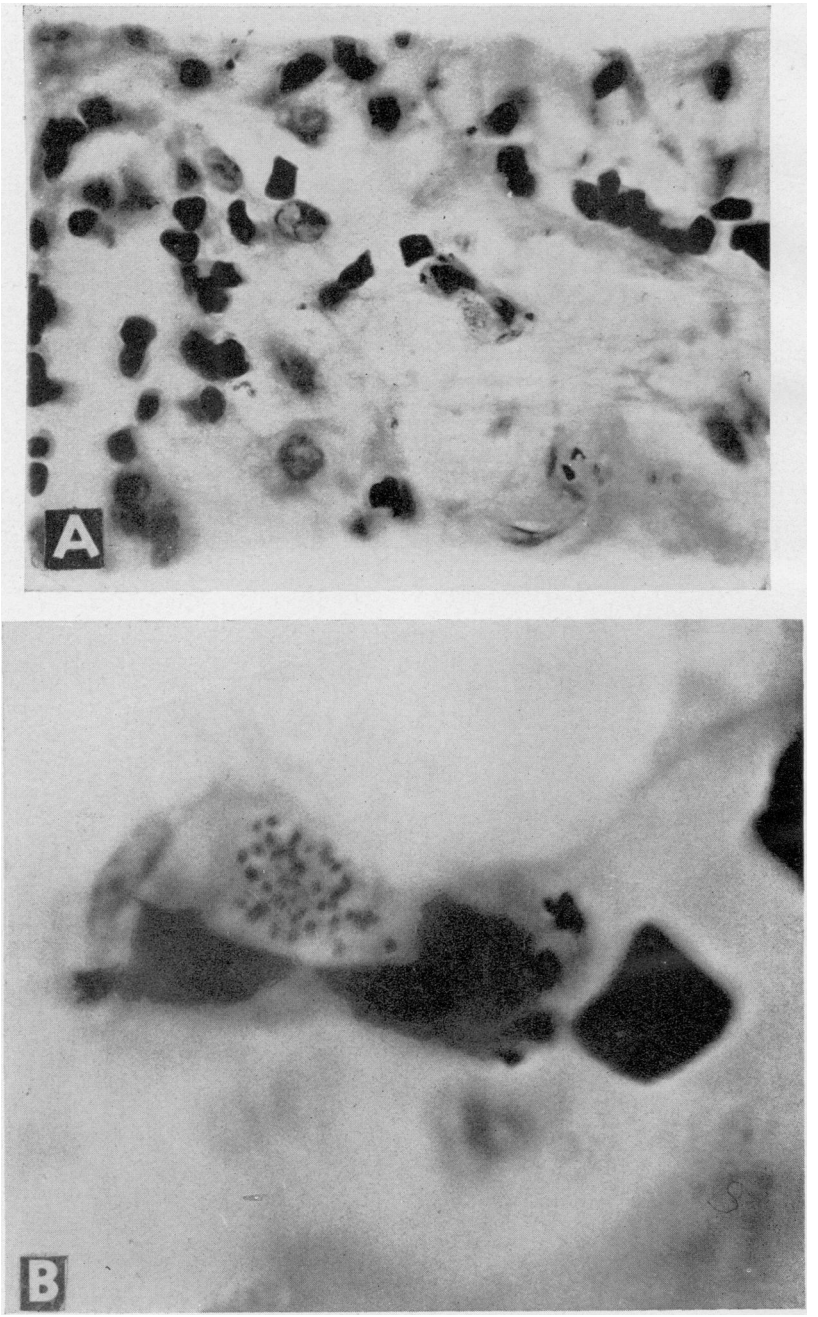


FIGURE 10.—Case 17. A. Intracellular cluster of minute basophilic bodies in pneumonic exudate; Romanowsky stain ×1,000. B. Same as A, greatly enlarged.

*Spleen.*—Follicles were small and were rimmed by a narrow zone of pale-stained large mononuclear cells. The sinuses were engorged and pulp interstitial zones filled with blood. Where identified the sinus reticulo-endothelial cells were swollen. A medium number of large phagocytic cells and plasma cells were seen in the pulp.

*Kidney.*—In numerous glomerular tufts, many capillary loops were filled with fibrin thrombi. The convoluted tubules exhibited ragged epithelium, and lumina were often dilated and contained granular material. Similar material in small amounts was seen in some glomerular capsules.

*Suprarenal gland.*—A few petechial hemorrhages were seen in the outer cortex. A mild periglandular plasma cell infiltration was noted. Occasionally in the subcapsular capillaries minute thrombi were observed. The medulla contained a few clusters of infiltrating lymphocytes.

*Urinary bladder.*—The epithelium was intact. There was sparse subepithelial mononuclear cell infiltration.

*Stomach.*—The glandular zone of the mucosa in an area less than 1 cm. in width exhibited recent necrosis and hemorrhage. This lesion did not extend deeper than the muscularis mucosae.

*Rectus muscle.*—Areas of hemorrhage were prominent. In areas muscle fibers were swollen, showed increased oxyphilia, and had lost all striations and nuclei. In these necrotic areas, proliferating fusiform cells were seen.

*Mammary gland.*—There was a slight cystic dilitation of the ducts, but no other changes were noted.

*Femoral nerve.*—With the exception of a few monocytes seen around some of the small blood vessels, no changes were observed.

*Thyroid gland.*—No lesions were seen.

*Lymph node, periaortic.*—Follicles were inactive, and sinuses were moderately widened but without unusual cell content.

*Vertebral bone marrow.*—The marrow was cellular. No abnormalities were observed.

*Brain.*—Cerebellar and cerebral cortex, corpus striatum, thalamus, pons, medulla, and cervical cord were studied. No inflammatory infiltrate or necrosis was seen.

*Bacteriology.*—As in case 17, at the time of the autopsy, using blood agar plates and thioglycollate broth heart blood, lungs, liver, spleen, meninges, and brain were cultured, and again were negative for pathogenic bacteria. No bacteria were seen in any of the smears of organs or in tissue sections.

With 2-mm. oil immersion objective much searching of lung slides finally revealed an extracellular cluster of minute basophil coccobacillary bodies. In a few swollen alveolar lining cells or large free cells minute faintly stained coccobacillary bodies were observed. No bodies were observed in the liver or spleen.

SUMMARY OF CASE 18

As seen in case 17, there was deep purplish-plum consolidation in varying proportions of the four major lobes. Sharp lines separated the involved from noninvolved lung. Again a predominantly large mononuclear cell, sero-sanguino-fibrinous alveolar exudate was found, and alveolar lining cells were hyperplastic. Interalveolar septa were slightly to moderately widened and with slight infiltration by mononuclear cells. Thrombosed septal capillaries were numerous. Thrombi frequently plugged small arteries, and a few small infarcts were seen. As in case 17, bronchioles and bronchi showed little change.

Associated changes were: serous pleural exudate, large hemorrhages and hyalin necrosis of rectus muscles, acute subcapsular hemorrhages and focal necroses of

liver, large mononuclear cells and swollen Kupffer cells in liver sinusoids, mild myocarditis, acute splenitis, a few capillary thromboses, and petechial hemorrhages of suprarenal cortex, thromboses in glomerular capillaries of kidney, and mild cystitis.

Nonrelated abnormalities were: calculus of pancreatic duct and multiple small uterine leiomyomata.

A few minute intra- and extracellular coccobacillary bodies were observed in pulmonary alveolar exudate.

## DISCUSSION

### PSITTACOSIS

Through the courtesy of Dr. R. D. Lillie[4] opportunity was given to study sections from several autopsies on cases of psittacosis. It is considered worth while to compare and contrast our 2 cases with psittacosis as seen in this material and as described by Dr. Lillie in his monograph (*3*), which deals with 9 cases studied by him and 43 cases collected from published autopsy reports.

In general, there appear to be many striking similarities in the pathologic changes in the two conditions, but in psittacosis there is definite tendency for the consolidation to be lobular in character even though a whole lobe is involved. Generally, the consolidation was referred to as gray or gray red and granular. In 3 of the 52 cases (cases 1, 25, and 41) a blue-red color is mentioned which may have been somewhat similar to the color we designated "purplish plum."

There was no reference to the sharply outlined zones of demarcation between involved and uninvolved parenchyma which was so impressive in both of our cases. In microscopic sections of the lungs of psittacosis cases the findings of many groups of air or serum-filled alveoli in otherwise consolidated sections was typical. This patchy distribution and the abrupt change in character of the alveolar exudate from field to field were not commonly seen in our sections. There was, though, definite similarity in (1) the composition of the alveolar exudate, (2) the hyperplasia of the alveolar lining cells, (3) the predominance of large mononuclear cells and relative absence of polymorphonuclear leucocytes in the alveolar exudate, (4) only slight widening and infiltration of interalveolar septa, and (5) little change in large bronchioles and bronchi.

Irregularly dispersed foci of coagulation necrosis were seen in the liver in several cases of psittacosis. Our second case had subcapsular foci of coagulation necrosis, but no necrosis was seen in the deeper areas. The swelling and phagocytic reaction of Kupffer cells appeared similar in the two diseases.

The splenic changes in psittacosis and in our cases were roughly comparable. In 1 case of psittacosis (case 50) occasional hyaline glomerular capillary occlusions were seen in the kidney. Many

[4] Chief, Pathology Laboratory, National Institute of Health.

thrombosed glomerular capillaries were observed in our second case. Waxy degeneration of rectus muscles was reported in 11 and hemorrhage into these muscles was seen in 10 cases of psittacosis. Hemorrhage was pronounced in the rectus muscles of our second case, and hyalin necrosis was present in both cases.

Thrombi of pulmonary arteries were described in three cases of psittacosis. There were a few thrombi in small pulmonary arteries in our first and in medium-sized pulmonary arteries in our second case. Subarachnoid hemorrhage as seen in our first case was not recorded in psittacosis, but in a few cases of that disease there were perivascular petechial hemorrhages and focal necrosis in the brain which were probably similar to those observed in our first case.

### Q FEVER

Also through the kindness of Dr. Lillie, slides from one case of Q fever (*4*) were studied and our cases compared with it directly as well as by use of the published pathologic report. In this case of Q fever the pneumonia was limited to one lobe and firm, gray consolidation was described. Microscopically there was some similarity in that the larger bronchioles and bronchi were relatively clear and polymorphonuclear leucocytes played little part in the alveolar exudate. In the lung of the Q fever case the alveolar exudate was more compact and had a much larger component of lymphocytes than in our cases. Also, in that condition the septa were more widened and considerably more infiltrated by lymphocytes and large mononuclear cells.

The liver in the case of Q fever did not show the Kupffer cell reaction seen in our cases, nor were the increased number of neutrophils found in the centrolobular sinusoids observed in our material. Splenic changes in the two conditions were roughly parallel.

### ATYPICAL OR VIRUS PNEUMONIA

The so-called virus pneumonia is rarely fatal, and only a few autopsy reports have been made.

Longcope (*5*) describes two deaths occurring in cases diagnosed "atypical" pneumonia. One was in a 38-year-old man who died on the seventh day of illness. Autopsy revealed "extensive bronchopneumonia and mitral stenosis and insufficiency." Small areas of consolidation were seen in lobes which at first seemed consolidated but later were found "rather filled with fluid than consolidated." A generalized severe acute bronchitis was described. The bronchial exudate was composed of "about half polymorphs and half round cells." Very few neutrophils were seen in alveolar exudate.

The other death was that of a 40-year-old man on the fourteenth day of illness. Rheumatic heart disease with aortic insufficiency

and pulmonary infarctions were contributing factors in his death. The areas of consolidation were small and peribronchial in distribution. A purulent bronchitis was present.

Kneeland and Smetana (*6*) reported the autopsy findings in a woman, age 47, who died from atypical pneumonia after a prolonged illness of 6 weeks' duration. A lobular consolidation was seen in several lobes. The older areas of consolidation showed greatly thickened alveolar septa. The copious exudation of large mononuclear cells occurring in an area of more recent consolidation and illustrated by photomicrograph resembles that seen extensively in our cases. In contrast with our cases, there were purulent tracheobronchitis, foci of fibrinopurulent pneumonia, and a necrotizing pulmonary arteritis resembling periarteritis nodosa. Focal necroses were described in the liver.

Dingle (*7*) and coauthors in their comprehensive review on the subject of atypical pneumonia referred to a death at Camp Claiborne. The autopsy report described a hemorrhagic interstitial bronchopneumonia and acute bronchitis. The only similarity to our cases seems to have been in the relative paucity of neutrophils in the alveolar exudate.

## SUMMARY

Detailed autopsy reports are made in two cases who died of pneumonitis. Each case exhibited similar lung changes, the most important of which were sharply defined purplish plum-colored consolidation, large mononuclear cell alveolar exudate, hyperplasia of alveolar lining cells, and slight or no involvement of bronchioles and bronchi. No bacterial etiology was established. A few clusters of intracytoplasmic, basophil, minute coccobacillary inclusions were seen in pulmonary alveoli in both and in a Kupffer cell in one case. These bodies appeared identical with "elementary bodies" seen in animals inoculated with organs from each case.

Pathologically these two cases in some respects resembled psittacosis but showed less in common with the one autopsy of Q fever which was reviewed and still less with the few reported autopsies of virus pneumonia.

## ACKNOWLEDGMENTS

Dr. E. L. Landry, New Iberia, La., the patients' physician; Dr. W. L. Treuting, director of preventive medicine, Louisiana State Health Department; and Dr. B. J. Olson, Division of Infectious Diseases, National Institute of Health, attended and assisted with each autopsy.

Appreciation is expressed to Dr. R. D. Lillie, Chief, Pathology Laboratory, National Institute of Health, and to members of his staff for examining slides from these cases and giving opinions. Fur-

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Medical Technician Rubye A. J. Wilson, laboratory, United States Marine Hospital, New Orleans, La., prepared the histologic sections and did the many special stains used in studying these cases. Her cooperation is appreciated.

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# AN EPIDEMIC OF A SEVERE PNEUMONITIS IN THE BAYOU REGION OF LOUISIANA

## IV. A PRELIMINARY NOTE ON ETIOLOGY[1]

By B. J. Olson, *Surgeon*, and C. L. Larson, *Passed Assistant Surgeon, United States Public Health Service*

Bacteriological studies were made of four cases of pneumonitis (cases 16, 17, 18, and 19). Throat washings were examined during their illness, and autopsy material was obtained from cases 17 and 18. The virus was isolated from all cases studied except case 19. Isolations were made from the throat washings of case 16 on the ninth day of illness and on cases 17 and 18 on the third day of illness. The same virus was recovered from cases 17 and 18 at autopsy. It was readily isolated in white mice by either the intraperitoneal or intranasal route of inoculation, and in guinea pigs by intraperitoneal injection of throat washings or tissue suspension. The symptoms and gross pathology in animals were similar to those produced by the psittacine group of viruses with the production of readily demonstrable elementary bodies in the smear of the spleen, liver, and lungs.

[1] From the Division of Infectious Diseases, National Institute of Health.

Owing to the fact that guinea pigs are highly susceptible and respond with a fatal infection, and that mice succumb when inoculated either intranasally, intraperitoneally, intracerebrally, intramuscularly, or subcutaneously with infectious material, it is suggested that we were dealing with a new etiological agent. A detailed report on the characteristics of the agent will be published at a later date.

## MOUSE PROTECTIVE ANTIBODIES IN HUMAN SERUMS FOLLOWING INJECTIONS WITH CHOLERA VACCINE[1]

By JAMES J. GRIFFITTS, *Passed Assistant Surgeon, United States Public Health Service*

Cholera has been confined, in recent years, to areas where it is considered endemic. However, the misfortunes of war have sent members of the armed forces into these areas in great numbers, and the danger of the spread of cholera to the military and civil populations must be considered. Among important safeguards is the vaccination against the disease of military personnel of the United States assigned to duty in regions where cholera is endemic.

This study was made to obtain data on the appearance and persistence of serum antibodies in man after injection with cholera vaccine

### PROCEDURE

Samples of blood were drawn from 34 white, male medical students 21 to 26 years of age, most of whom were 22 or 23 years old. None of the group gave a history of cholera vaccination, nor had any ever been in endemic or recently epidemic areas.

Following the withdrawal of blood samples each student was given an injection subcutaneously of 0.5 cc. and, after 1 week, 1.0 cc. of a vaccine prepared at the National Institute of Health.

Blood samples were taken from the entire group 2 weeks, 3 months, 6 months, 12 months, and 18 months after these injections were completed. In addition, samples were taken from 16 members of the group 1 week and again 1 month after the last injection.

Following the bleeding at 6 months, 16 students received an additional 1.0 cc. of vaccine. Seven weeks later these students were bled, as were an equal number of volunteers who had not received additional vaccine.

Thirteen months after the initial course of vaccine all but 7 of the group were given an additional 1.0 cc. injection of the vaccine.

*The vaccine.*—The procedure recommended by the Biologics Control Laboratory of the National Institute of Health was followed in the preparation of cholera vaccine. Inaba (NIH 35A3) and Ogawa

[1] From Biologics Control Laboratory, National Institute of Health.

(NIH 41) strains of *V. cholerae* were grown separately on meat infusion agar for 24 hours at 37° C. The growth was washed off with salt solution (containing 0.5 percent phenol) and suspensions equal to 8,000 million organisms per cc. were made by matching with turbidity standards. Equal amounts of Inaba and Ogawa vibrio suspensions were pooled. Sterility tests and safety tests were made and the vaccine was assayed in mice for potency. This vaccine was equivalent to the vaccines supplied to the armed forces of the United States.

## REACTIONS

*Local.*—Twenty-five of 34 men had tenderness about the site of the first injection, 4 of whom stated that the local pain was moderately severe and accompanied by aching in the arm injected. The remaining 9 had no soreness. After the second injection, local reactions were about the same, 22 having moderate tenderness. The site of injection was usually surrounded by an area of redness and slight edema 3 to 6 cm. in diameter. Ten individuals had local reactions extending 10 to 12 cm. after the first dose.

*Systemic.*—Ten men complained of headache, usually appearing 2 to 5 hours after the first injection of vaccine and lasting a few hours. After the second dose, 5 had moderately severe headache, 3 of whom had similar symptoms after the first injection. Three students reported slight fever (temperatures were not recorded) with accompanying chilliness after the first dose. Three others complained of slight muscular aching. In one instance slight axillary gland enlargement with tenderness was reported.

The reactions are summarized in table 1. In general, the extent of reactions was more moderate after the second dose of vaccine. Reactions were not severe as no student was absent from class because of reaction and no systemic symptom persisted longer than 24 hours, nor local discomfort longer than 48 hours. Following additional doses at 6 and 13 months, respectively, local reactions were slight and no systemic reaction was reported.

TABLE 1.—*Summary of reactions in 34 individuals given cholera vaccine*

| Symptom or sign | Frequency of occurrence | |
|---|---|---|
| | After first injection | After second injection |
| Tenderness at site of injection | 25 | 22 |
| Redness and edema: | | |
| 3 to 6 cm. diameter | 24 | 30 |
| Over 6 cm. diameter | 10 | 4 |
| Headache | 10 | 5 |
| Fever | 3 | 0 |
| Muscular aching | 3 | 0 |
| Axillary gland enlargement | 1 | 0 |

METHODS

Samples of blood were drawn and serums separated under aseptic conditions. Immediately after separation serums were stored at 0° C. and tests begun within 3 days of bleeding were completed in 1 week.

*Mouse protection tests.*—These procedures were patterned after the methods described by Siler and others, of the United States Army Medical School, for serum protection tests against *E. typhosa* (*1*). Equal numbers of male and female white Swiss mice of the regular National Institute of Health strain, weighing 10 to 13 grams, were used. Twenty-five hundredths cc. of the individual serum diluted 1:2.5 with sterile physiologic salt solution was injected intraperitoneally into each of 30 mice. Each mouse thus received 0.1 cc. of serum. One hour after serum was given, 6 groups of 5 mice each received varying doses of living vibrios in mucin by intraperitoneal injection. Three groups of mice received the Inaba strain (NIH 35A3) in doses of approximately 50,000,000, 5,000,000, and 500,000 vibrios, respectively. The other three groups received three comparable doses of the Ogawa strain (NIH 41). The test strains were those used in preparing vaccine and on each day of testing a fresh culture, preserved by drying *in vacuo* from the frozen state in sterile milk, was used.

Test doses of vibrios were prepared from 5- to 6-hour-old meat infusion agar cultures, the basic dilution being a saline suspension of vibrios having a turbidity equal to that of 500 parts per million of silica standard (*2*). One part of this dilution in 9 parts of 5 percent mucin suspension represented the $10^{-1}$ test dose (50,000,000 vibrios). Serial tenfold dilutions were prepared in normal saline, test doses being tenfold dilutions in mucin from saline suspensions one dilution lower. Pour plate colony counts of the $10^{-7}$ saline dilutions on each day of testing gave on the average approximately 100 organisms per cc.

One-half cc. amounts of mucin-suspended organisms were injected intraperitoneally, the dilutions $10^{-1}$, $10^{-2}$, $10^{-3}$ being given mice which had received serum while control animals were given the dilutions $10^{-5}$, $10^{-6}$, $10^{-7}$, and $10^{-8}$. On the first few tests, serums of unvaccinated individuals were used in virulence titrations of the vibrios, and since no appreciable difference was found between these and subsequent titrations in mice without previous serum injection, the majority of virulence tests were made without serum injections. Separate tests were performed with the Inaba and Ogawa strains of *V. cholerae*. Animals were observed for 72 hours.

The mucin vehicle was prepared by suspending 100 gm. of granular mucin in 2,000 cc. of distilled water. This suspension, after straining

through 6 to 8 thicknesses of gauze and adjusting to pH 7.4 with 0.1 N. sodium hydroxide, using bromthymol blue as an indicator, was autoclaved in small flasks at 15 pounds pressure for 30 minutes.

Fifty percent end points (*3*) were calculated on the basis of the number of lethal doses against which mice were protected by serums. A sample protocol (table 2) shows results of tests at various dates, including virulence titration and the number of lethal doses against which 50 percent of mice were protected by the injection of serum No. 10.

TABLE 2.—*Protocol showing results of mouse protection tests with serum No. 10 at various intervals before and after vaccination. Test strain: Inaba (35A3)* Vibrio cholerae

| Date of bleeding | Amount of serum per mouse | Dilution test organism | Number of vibrios | Number of mice | Deaths | | | | Number of lethal doses resisted [1] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 24 hours | 48 hours | 72 hours | Total | |
| Aug. 21, 1942 (before vaccination). | 0.1 cc | $10^{-1}$ | 62,000,000 | 5 | 5 | ---- | ---- | 5 | Less than 3,000. |
| | | $10^{-2}$ | 6,200,000 | 5 | 5 | ---- | ---- | 5 | |
| | | $10^{-3}$ | 620,000 | 5 | 4 | 1 | ---- | 5 | |
| | Controls (0.1 cc. normal serum) | $10^{-4}$ | 6,200 | 5 | 4 | 1 | ---- | 5 | Dilution = One 50 percent LD = 1/3,160,000. |
| | | $10^{-6}$ | 620 | 5 | 3 | 0 | 0 | 3 | |
| | | $10^{-7}$ | 62 | 5 | 1 | 1 | 0 | 2 | |
| | | $10^{-8}$ | 6 | 5 | 0 | 0 | 0 | 0 | |
| Sept. 11, 1942 (2 weeks after last dose). | 0.1 cc | $10^{-1}$ | 72,000,000 | 5 | 0 | 0 | 0 | 0 | Greater than 144,000. |
| | | $10^{-2}$ | 7,200,000 | 5 | 0 | 0 | 0 | 0 | |
| | | $10^{-3}$ | 72,000 | 5 | 0 | 0 | 0 | 0 | |
| | Controls (no serum). | $10^{-5}$ | 7,200 | 5 | 3 | 1 | 0 | 4 | Dilution = One 50 percent LD = 1/1,440,000. |
| | | $10^{-6}$ | 720 | 5 | 3 | 0 | 0 | 3 | |
| | | $10^{-7}$ | 72 | 5 | 1 | 0 | 0 | 1 | |
| | | $10^{-8}$ | 7 | 5 | 0 | 0 | 0 | 0 | |
| Nov. 20, 1942 (3 months after last dose). | 0.1 cc | $10^{-1}$ | 43,000,000 | 5 | 2 | 0 | 0 | 2 | 200,000. |
| | | $10^{-2}$ | 4,300,000 | 5 | 1 | 0 | 0 | 1 | |
| | | $10^{-3}$ | 430,000 | 5 | 0 | 1 | 0 | 1 | |
| | Controls (no serum). | $10^{-5}$ | 4,300 | 5 | 2 | 2 | 0 | 4 | Dilution = One 50 percent LD = 1/3,160,000. |
| | | $10^{-6}$ | 430 | 5 | 3 | 0 | 0 | 3 | |
| | | $10^{-7}$ | 43 | 5 | 2 | 0 | 0 | 2 | |
| | | $10^{-8}$ | 4 | 5 | 0 | 1 | 0 | 1 | |

[1] 50 percent end points.

The $10^{-1}$ dilution was the largest dose of the series which could be used to show protection even when hyperimmune rabbit serum was given to the mice. Ten times this dose was too toxic for mice. Because of this limiting factor, it was supposed that greater differences in the mouse protective ability of serums might be shown by challenging mice given various amounts of serum with a constant test dose of organisms. Since the optimal test dose was not known, 3 doses of organisms were used with each serum dilution. Thus, in addition to the lethal dose titrations described above, data were obtained by titering the serums of 16 individuals taken before the injections and one week, 2 weeks, 1 month, 3 months, and 6 months after the injections. Four pools of 4 serums each were made, and each pool was diluted so that 3 groups of 15 mice each received 0.1 cc., 0.01 cc., and

0.001 cc. of serum, respectively. The groups of mice were further divided into units of 5 mice each and the test doses $10^{-1}$, $10^{-2}$, and $10^{-3}$ were injected. The dilution of serum which protected 50 percent of mice was calculated by the 50 percent end point method. A sample protocol is shown in table 3.

TABLE 3.—*Protocol showing results of mouse protection tests with serum pool No. 1. Three dilutions of the serum pool of each bleeding are tested with three test doses of* Vibrio cholerae, *Inaba strain 35AS*

| Date of bleeding | Test dilution of vibrios | Approximate number of vibrios | Amount of serum per mouse (cc.) | Number of mice injected | Survivals | Deaths | Calculated 50 percent end point o serum |
|---|---|---|---|---|---|---|---|
| Aug. 21, 1943 (before vaccination). | $10^{-1}$ | 59,000,000 | 0.1 | 5 | 0 | 5 | Greater than 0.1 cc. |
| | $10^{-2}$ | 5,900,000 | | 5 | 0 | 5 | |
| | $10^{-3}$ | 590,000 | | 5 | 0 | 5 | |
| Sept. 4, 1943 (1 week after last dose). | $10^{-1}$ | 59,000,000 | 0.1 | 5 | 3 | 2 | 0.068 cc. |
| | | | 0.01 | 5 | 0 | 5 | |
| | | | 0.001 | 5 | 0 | 5 | |
| | $10^{-2}$ | 5,900,000 | 0.1 | 5 | 5 | 0 | |
| | | | 0.01 | 5 | 2 | 3 | 0.01 cc. |
| | | | 0.001 | 5 | 1 | 4 | |
| | $10^{-3}$ | 590,000 | 0.1 | 5 | 5 | 0 | 0.0014 cc. |
| | | | 0.01 | 5 | 5 | 0 | |
| | | | 0.001 | 5 | 2 | 3 | |
| | $10^{-4}$ | 5,900 | No serum | 5 | 0 | 5 | |
| | $10^{-5}$ | 590 | | 5 | 1 | 4 | |
| | $10^{-6}$ | 59 | | 5 | 3 | 2 | |
| | $10^{-7}$ | 6 | | 5 | 5 | 0 | |

*Agglutination reactions.*—Duplicate sets of serum dilutions were made to determine the agglutinin titers for the Inaba and Ogawa strains of *V. cholerae*. The serums were diluted in threefold steps from 1:20 through 1:4860.

Suspensions of living 6-hour agar-grown cholera vibrios, in normal saline, were used as antigens. The turbidity of the suspensions was equal to that of 300 parts per million of the silica standard. Equal parts of antigen and serum dilutions were mixed in a small test tube, agitated, and incubated at 37° C. for 2 hours. Reactions were read immediately and again after 22 hours at 5° C. Readings were made without lenses, and a positive reaction was one in which macroscopic clumps persisted after shaking the contents of the tube. No attempt was made to distinguish "O" and "H" agglutinins. High titered "O" rabbit serums were used as positive controls while saline suspensions of organisms served to rule out spontaneous agglutination of the antigens.

## RESULTS

*Mouse protection tests.*—Serums of 34 students before vaccination showed a uniform lack of mouse protective substances against the three test doses of vibrios used, and an absence of agglutinins for either strain of vibrio. The degree of immunity exhibited by serums before and after vaccination is summarized in table 4. Immunity

TABLE 4.—*Degree of protection afforded mice against* V. cholerae *by 0.1 cc. of individual serums before and after vaccination*

| Time of bleeding | Vaccination history | Number of serums | Number of serums—distributed by degree of protection against *V. cholerae* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Inaba strain | | | | | | | | | Ogawa strain | | | | | | | |
| | | | 500,000 | | | 5,000,000 | | | 50,000,000 | | | 500,000 | | | 5,000,000 | | | 50,000,000 | | |
| | | | N | P | C | N | P | C | N | P | C | N | P | C | N | P | C | N | P | C |
| Before | No vaccine | 34 | 33 | 1 | 0 | 33 | 1 | 0 | 34 | 0 | 0 | 33 | 1 | 0 | 33 | 1 | 0 | 34 | 0 | 0 |
| 2 weeks after | 0.5 cc. and 1.0 cc. at 1 week interval | 34 | 0 | 1 | 33 | 0 | 0 | 34 | 3 | 11 | 20 | 0 | 1 | 33 | 0 | 0 | 34 | 6 | 10 | 18 |
| 3 months after | | 34 | 0 | 2 | 32 | 0 | 1 | 33 | 14 | 16 | 4 | 0 | 0 | 34 | 0 | 4 | 30 | 3 | 22 | 9 |
| 6 months after | | 34 | 0 | 5 | 29 | 2 | 8 | 24 | 18 | 11 | 5 | 0 | 4 | 30 | 2 | 6 | 26 | 0 | 12 | 22 |
| 7¾ months after | Additional dose at 6 months | 16 | 0 | 2 | 14 | 0 | 3 | 13 | 5 | 6 | 5 | 1 | 0 | 15 | 0 | 1 | 15 | 6 | 7 | 3 |
| | No additional dose | 16 | 1 | 1 | 14 | 1 | 4 | 11 | 6 | 7 | 3 | 0 | 1 | 15 | 0 | 3 | 13 | 8 | 5 | 3 |
| | Total | 32 | 1 | 3 | 28 | 1 | 7 | 24 | 11 | 13 | 8 | 1 | 1 | 30 | 0 | 4 | 28 | 14 | 12 | 6 |
| 12 months after | Additional dose at 6 months | 16 | 1 | 2 | 13 | 0 | 3 | 13 | 4 | 12 | 0 | 1 | 4 | 11 | 0 | 3 | 13 | 1 | 9 | 6 |
| | No additional dose | 16 | 0 | 3 | 13 | 0 | 3 | 13 | 7 | 7 | 2 | 1 | 0 | 15 | 0 | 5 | 11 | 7 | 6 | 3 |
| | Total | 32 | 1 | 5 | 26 | 0 | 6 | 26 | 11 | 19 | 2 | 2 | 4 | 26 | 0 | 8 | 24 | 8 | 15 | 9 |
| 18 months after | No additional vaccine | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 3 | 0 | 1 | 2 | 1 | 1 | 1 |
| | Additional dose at 6 months only | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 2 | 2 | 0 | 0 | 4 | 0 | 1 | 3 | 0 | 4 | 0 |
| | Additional dose at 13 months only | 14 | 0 | 0 | 14 | 0 | 3 | 11 | 4 | 8 | 2 | 0 | 2 | 12 | 0 | 9 | 5 | 5 | 7 | 2 |
| | Additional doses at 6 and 13 months | 12 | 0 | 0 | 12 | 0 | 0 | 12 | 0 | 8 | 4 | 0 | 1 | 11 | 0 | 2 | 10 | 4 | 3 | 5 |
| | Total | 33 | 0 | 0 | 33 | 0 | 3 | 30 | 4 | 20 | 9 | 0 | 3 | 30 | 0 | 13 | 20 | 10 | 15 | 8 |

N=No protection (4 or 5 of 5 mice died).
P=Partial protection (2 or 3 of 5 mice died).
C=Complete protection (4 or 5 of 5 mice survived).

was designated as complete when none, or 1, of the 5 mice tested died; partial in those wherein 2 or 3 died; and immune substances were judged absent when 4 or 5 of the test animals died.

Two weeks after the second dose of vaccine the majority of serums protected mice against even the largest challenge dose.

At 3 months and at 6 months after vaccination all serums protected mice against the smaller test doses while there was a definite decrease in the number of serums protecting mice against the largest test dose.

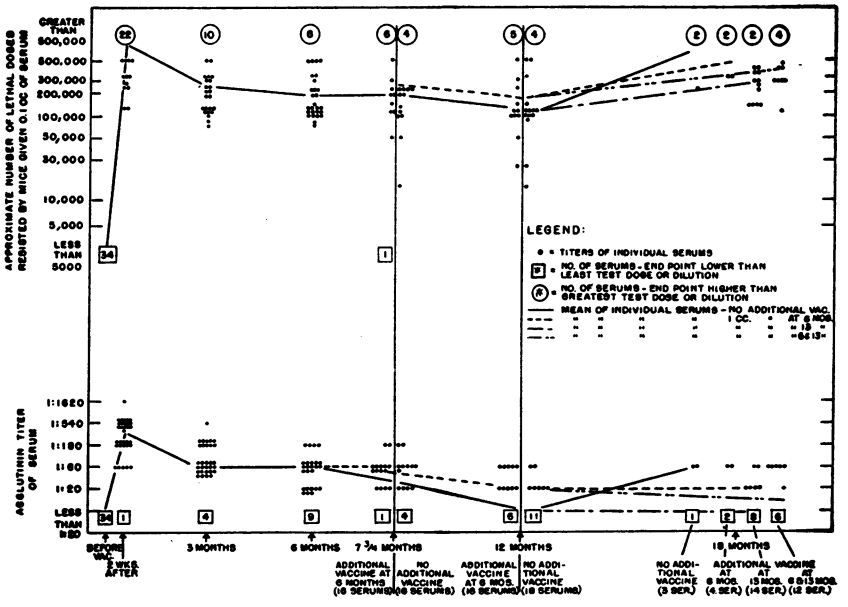


FIGURE 1.—Mouse protective titers and agglutinins of serums of individuals injected with cholera vaccine

Of those receiving an additional dose of vaccine at 6 months, a slightly greater number showed partial or complete protection 7 weeks and 6 months later than those getting no additional vaccine.

A greater proportion of the serums of those receiving two additional doses of vaccine completely or partially protected mice than serums of those given but one additional dose at 13 months after the initial course of vaccine. However, three individuals who received no additional vaccine maintained a high level of mouse protective antibodies for 18 months.

The number of lethal doses against which mice were protected by the injection of 0.1 cc. of the individual serums was distributed as shown in figure 1. Agglutinin titers for each serum are represented in the lower part of the figure.

Before vaccination, end points were not reached as serums did not protect mice against the $10^{-3}$ test dose, while after vaccination end points frequently were not reached as many serums gave complete

protection against the largest test dose. As shown in figure 1 the mean of serums prior to vaccination showed no protection against 5,000 lethal doses of the Inaba strain. Two weeks after vaccination the mean rose above the 500,000 lethal dose level; at 3 months it had fallen to approximately 300,000 lethal doses, and at 6 months to approximately 200,000 lethal doses.

Serums of those given an additional dose of vaccine at 6 months showed a very slight increase in protective ability 7 weeks later and 6 months later. The revaccinating doses appeared to cause a slightly higher level of immunity for the entire group as reflected in the mean titers yet, perhaps due to individual variations, three serums from those who received no additional dose of vaccine were at as high a level as the others.

Differences in the concentration of protective substances in serums as indicated by the number of lethal doses resisted by mice were further confirmed by serum dilution studies of the 4 pools of serum (table 5).

TABLE 5.—*Pooled serums. Amount of serum protecting 50 percent of mice against 3 test doses of the Inaba strain of* Vibrio cholerae, *at various times before and after vaccination. Each pool consists of serums from the same 4 individuals at each bleeding*

| Time of bleeding in relation to vaccination | Amount of serum (cc.) required to protect 50 percent of mice against test doses of vibrios | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test dose=5,000 LD [1] | | | | Test dose=50,000 LD [1] | | | | Test dose=500,000 LD [1] | | | |
| | Pool 1 | Pool 2 | Pool 3 | Pool 4 | Pool 1 | Pool 2 | Pool 3 | Pool 4 | Pool 1 | Pool 2 | Pool 3 | Pool 4 |
| Before | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 | >0.1 |
| 1 week after | 0.0014 | 0.006 | 0.004 | 0.001 | 0.01 | 0.006 | 0.024 | 0.0014 | 0.068 | 0.032 | 0.032 | 0.032 |
| 2 weeks after | <0.001 | <0.001 | <0.001 | <0.001 | 0.006 | 0.006 | 0.002 | 0.032 | >0.1 | >0.1 | >0.1 | 0.068 |
| 1 month after | 0.001 | 0.001 | 0.001 | 0.002 | 0.005 | 0.005 | 0.003 | 0.032 | >0.1 | >0.1 | 0.032 | 0.068 |
| 3 months after | 0.01 | 0.032 | 0.014 | 0.004 | 0.032 | 0.032 | 0.042 | 0.026 | 0.068 | >0.1 | >0.1 | >0.1 |
| 6 months after | 0.032 | 0.032 | 0.014 | 0.042 | 0.068 | 0.068 | 0.042 | 0.042 | 0.068 | >0.1 | >0.1 | >0.1 |

[1] 50 percent lethal doses.

Less serum was required to protect mice against each test dose of vibrios during the period from 1 week to 1 month after vaccination than at 3 or 6 months. At this latter date, however, protection was afforded mice against large numbers of vibrios by as little as 0.014 to 0.068 cc. of serum. Of the 16 individuals represented in the 4 pools above, some received additional vaccine, others did not, and these pools could not be compared at 12 and 18 months. However, separate pools were made of the serums of (*a*) 3 individuals who received the first two vaccine injections, (*b*) 4 students who received an additional dose at 6 months only, (*c*) 14 students who received an additional dose at 13 months only, and (*d*) 12 students who received additional doses

at 6 and 13 months, respectively. The amounts of serum of each of the above groups necessary to protect 50 percent of mice against approximately 50,000 lethal doses were (*a*) 0.05 cc., (*b*) 0.043 cc., (*c*) 0.036 cc., (*d*) 0.039 cc. The concentration of mouse protective antibodies thus appeared to be greater in those receiving the additional dose at 13 months although differences were very slight.

No material variations in results were noted in tests using the Inaba or Ogawa strains except that the Ogawa strain was consistently more virulent for mice, i. e., fewer organisms were required to kill control animals.

*Agglutination reactions.*—Agglutinins absent before vaccination appeared in high titer 1 week after vaccination in the serums of 15 individuals (table 6). In the entire group titers were highest at 2 weeks, the mean of the serums being 1:360 dilution when tested with the Inaba strain. At 3 and 6 months after vaccination the mean was a dilution of 1:60. At 12 months, in spite of the additional dose of vaccine being given to half of the group, the titers dropped, the mean being 1:20 in those receiving an additional dose of vaccine at 6 months and less than 1:20 among others. After 18 months the level for the whole group was quite low (fig. 1).

TABLE 6.—*Serum agglutinin titers in individuals vaccinated with cholera vaccine*

| Time of bleeding | Number of serums | Number of serums distributed according to titer | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Against Inaba strain | | | | | | Against Ogawa strain | | | | | |
| | | Less than 1:20 | 1:20 | 1:60 | 1:180 | 1:540 | 1:1620 | Less than 1:20 | 1:20 | 1:60 | 1:180 | 1:540 | 1:1620 |
| Before vaccination | 34 | 34 | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 |
| 1 week after | 15 | 1 | 0 | 1 | 6 | 6 | 1 | 1 | 0 | 0 | 2 | 6 | 6 |
| 2 weeks after | 34 | 1 | 0 | 5 | 11 | 16 | 1 | 0 | 0 | 2 | 6 | 12 | 14 |
| 1 month after | 15 | 0 | 0 | 0 | 8 | 7 | 0 | 0 | 0 | 1 | 8 | 5 | 1 |
| 3 months after | 34 | 4 | 0 | 19 | 10 | 1 | 0 | 8 | 1 | 11 | 13 | 1 | 0 |
| 6 months after | 34 | 9 | 8 | 13 | 4 | 0 | 0 | 8 | 7 | 14 | 5 | 0 | 0 |
| 7¾ months after: | | | | | | | | | | | | | |
| Additional 1 cc. vaccine at 6 months | 16 | 1 | 4 | 9 | 2 | 0 | 0 | 0 | 1 | 9 | 6 | 0 | 0 |
| No additional vaccine | 16 | 4 | 4 | 6 | 2 | 0 | 0 | 6 | 1 | 6 | 3 | 0 | 0 |
| Total | 32 | 5 | 8 | 15 | 4 | 0 | 0 | 6 | 2 | 15 | 9 | 0 | 0 |
| 12 months after: | | | | | | | | | | | | | |
| Additional vaccine at 6 months | 16 | 6 | 5 | 5 | 0 | 0 | 0 | 6 | 5 | 5 | 0 | 0 | 0 |
| No additional vaccine | 18 | 11 | 5 | 2 | 0 | 0 | 0 | 11 | 4 | 3 | 0 | 0 | 0 |
| Total | 34 | 17 | 10 | 7 | 0 | 0 | 0 | 17 | 9 | 8 | 0 | 0 | 0 |
| 18 months after: | | | | | | | | | | | | | |
| No additional vaccine | 3 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| Additional vaccine at 6 months | 4 | 2 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 |
| Additional vaccine at 13 months | 14 | 8 | 4 | 2 | 0 | 0 | 0 | 8 | 2 | 4 | 0 | 0 | 0 |
| Additional vaccine at 6 and 13 months | 12 | 6 | 1 | 5 | 0 | 0 | 0 | 6 | 3 | 3 | 0 | 0 | 0 |
| Total | 33 | 17 | 5 | 11 | 0 | 0 | 0 | 16 | 6 | 11 | 0 | 0 | 0 |

Considering the group as a whole, agglutinin titers generally paralleled the mouse protective titer for at least 6 months. At 12 months the agglutinins dropped more sharply than mouse protective titers. Individual serums frequently showed a lack of correlation between height of agglutinins and protective antibodies.

## DISCUSSION

Previous immunological studies on the effect of cholera vaccine in man employing serum agglutination and bactericidal tests have been made (*4*, *5*, *6*) and results reported here are in agreement with those as far as early appearance and persistence of agglutinins are concerned. Agglutinin titers in the present study were higher than is usually reported, probably owing to the use of living vibrios as antigens in the tests.

It appears from this study that mice may be of definite value in demonstrating specific protective substances in serum against cholera organisms and that these substances may be present in the absence of agglutinins for the vibrio. The significance of the level of protective antibodies in relation to the ability of the individual to resist cholera infection following exposure is, of course, not known. Serum protective antibodies appear within 1 week and are quite concentrated for 1 month after vaccination, a finding that should warrant the use of vaccine in the face of threatening cholera epidemics. The apparent lack of appreciable increase in the mouse protective antibody level after revaccination at 6 months does not follow the pattern seen in diphtheria or tetanus revaccination studies, in which a definite sharp rise in antibodies has been demonstrated (*7*, *8*). This may be due to the limits of the test procedure, to the possibility that a sharp brief rise may have occurred and subsided in 7 weeks, or that no additional stimulus to antibody formation resulted from the revaccinating dose.

## SUMMARY

A mouse protection test of human serums against *V. cholerae* is described.

Mouse protective substances appeared in the serum of human volunteers 1 week following vaccination. These antibodies were present at least 18 months after vaccination and were more concentrated in serums at 1 week and 1 month than at 3 or 6 months.

Agglutinins appear 1 week after vaccination, remain at high titer (1:180–1:1620) for 1 to 2 weeks and then decline, the majority of serums having low titers at 6 months, 1 year, and 18 months. In this study no definite correlation between height of agglutinin and mouse protective titers could be made.

ACKNOWLEDGMENTS

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## THE REARING OF STERILE ADULT *ANOPHELES* [1]

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Trager (*1*) obtained sterile *Aedes aegypti*, larvae and adults, in a medium consisting of autoclaved liver extract and brewers' yeast. Similar results have not been reported for *Anopheles* in this or other sterile medium. Barber (*2*) reared *Anopheles* in various media containing but a single species of living microorganism. Mature *Anopheles* were obtained in pure cultures of an infusorian, a yeast, an alga, and a bacterium.

The aim of the present work was to determine if in any of these pure cultures the microorganism dies out leaving sterile adult *Anopheles*; further, if such proved to be the case, whether such sterile adults could be obtained in a sufficiently robust condition and in quantities adequate for use in experiments.

The medium used in most of the present work was basically that of the yeast and liver extracts of Trager. This medium was modified somewhat to suit the microorganisms grown with the larvae, since many trials showed that the medium alone did not regularly bring the anopheline larvae even to the fourth instar. The basic medium could be made very simply: To tap or distilled water was added by weight 0.7 percent of Lilly liver extract and 1.0 percent of Harris brewers' yeast powder. The tap water of Memphis, Tenn., obtained from deep

[1] From the Office of Malaria Investigations, National Institute of Health.

wells, served as well as did distilled water. This mixture was brought to boiling over the free flame, with vigorous stirring to avoid scorching. Enough sodium hydroxide was added to bring the pH to approximately 6.5. An exact adjustment of the pH did not seem to be essential for the microorganisms employed. These grew well in a medium varying from pH 6.0 to pH 7.0. The addition of certain ingredients seemed to favor the growth of ciliates, for example, 2 gm. per liter of Difco blood serum. This powder was added gradually while the basic medium was boiling hot and was well stirred in, with the result that particles of coagulated blood serum remained suspended in the liquid. Addition of 1 percent of dextrose improved the growth of some of the organisms. To a flask containing 350 cc. of medium was usually added at least 20 cc. of fine, clean sand or earth in order to insure the presence of various inorganic constituents. After autoclaving, the addition of about 1 percent of sterile blood or blood serum, although not essential, seemed to improve the medium.

To the autoclaved medium sterilized eggs of anophelines were added, together with a pure culture of the microorganism which was to serve as larval food. Among various bacteria and infusorians employed, certain of the ciliates proved to be most successful. They were less liable than bacteria to produce pellicles or otherwise to modify the medium so as to render it less fit for larvae. Two species (or possibly strains) of *Glaucoma* gave very good results. I also used *Tetrahymena geleii*.[2] All of these ciliates were "pure line" cultures isolated by means of the pipette.

The addition of a pure culture of a nonmotile alga, a species of *Ankistrodesmus*, did not materially improve the medium.

The mosquito eggs were sterilized on a cloth held in a perforated spoon as described by Barber (*2*). The eggs were washed from the filter paper on which they were laid into the cloth on the spoon. They were then treated for a few seconds with 70 percent alcohol or other reagent to remove the air, then for about 15 minutes with White's solution (mercuric chloride, 0.25 gm.; sodium chloride, 6.5 gm.; hydrochloric acid, 1.25 cc.; ethyl alcohol, 250 cc.; distilled water, 750 cc. (*1*)). This solution was dripped over and between the eggs for about 15 minutes. The White's solution was then washed away by dripping with sterile distilled or tap water for about 5 minutes, and the cloth and eggs removed from the spoon and dried for about 15 minutes between layers of wire gauze with asbestos centers, previously sterilized in the Bunsen flame. Dried eggs float and a larger proportion of them hatch than when sunken.

By means of a moistened platinum spatula the eggs were taken up and distributed in test tubes containing beef broth or other medium suitable for determining the presence of bacteria. Usually they were

[2] The author is indebted to Prof. G. W. Kidder, of Brown University, for identification of this ciliate.

kept for 2 days in the broth until hatched, and only the test tubes containing sterile larvae were subsequently transferred to flasks. Sometimes the dried eggs were transferred directly to the breeding flasks and their sterility determined by later tests.

The eggs were usually sterilized in batches of several hundred and 50 or more transferred to each test tube. The proportion hatching in different batches varied from 30 percent to 1 percent or less, a part of the eggs being killed in sterilization.

As a rule, eggs laid the night before sterilization were employed, usually from a race maintained in an insectary. In a few batches eggs from wild mosquitoes were used. All were *Anopheles quadrimaculatus;* however, some limited experiments with other species indicated that these could be reared as well.

Mosquito cultures were usually done in Erlenmeyer flasks of from 500 cc. to 3 liters capacity. Test tubes proved unfavorable, as might be expected from the feeding habits of *Anopheles*, where wide surfaces are advantageous. Cultures were kept at room temperature or in the insectary at a temperature of about 25° C.

The adults reared in these cultures were usually large and strong when proper conditions were maintained. Their longevity could be increased by feeding on sterile defibrinated blood plus a little honey. Feeding of adults on defibrinated blood thus treated may be employed in the insectary as a substitute for feeding on the living animal, and fertile eggs are also obtained by this means. It is theoretically possible to rear a batch of *Anopheles* in a flask, to feed them there, and to obtain fertilization and oviposition: thus to maintain a flask colony.

The following tests of contamination or sterility were employed: Of the fluid in which the mosquitoes had reached the pupal or adult stage large samples were cultured both aerobically and anaerobically for contaminants. Adult mosquitoes were likewise cultured to determine the presence of bacteria. Tests were made in media favorable for the growth of the infusorian. Many such tests were done in various media and under various conditions of growth. Tests of sterility were continued in order to detect the presence of any slow-growing contaminants. Smears were made of both abdominal and thoracic contents of adult mosquitoes. It seemed definitely proved that adult mosquitoes were obtained free from any kind of microorganism, the ciliate being too sensitive to drying or otherwise rendered unable to carry over into the adult mosquito. If any resistant forms of the ciliate ever developed, they did not appear in medium used.

Not all cultures developed an abundance of strong mosquitoes capable of flight. There was comparatively little difficulty in obtaining large, apparently healthy larvae of the fourth instar, but not uncommonly a portion would die after reaching this or the pupal stage, or would emerge on the surface of the liquid but be unable to

fly afterwards. Many experiments were done to insure larger batches of adults. Various media were employed, including the sterile bodies of adult *Anopheles* heated and unheated. It appeared that the quantity as well as the quality of food, especially that of larger larvae, was a very important factor in development of anophelines.

It was doubtful if waste products of metabolism played an important part in inhibiting growth. Cultures in which a batch of adults had been successfully reared were reinoculated and again gave good results.

But in spite of some failures, many batches of strong, flying, sterile adults were obtained, sometimes 10 or more per flask, a sufficient number for experimental purposes.

### CULTURES OF LARVAE WITH HEAT-KILLED CILIATES

At least two of the species of ciliate employed were killed when exposed to a temperature of 40° C. for 5 minutes. Many attempts were made to grow larvae to maturity in heat-killed cultures. Growth was better than in any other wholly sterile medium, often proceeding rapidly to the fourth instar; but good pupae or adults were never obtained. It is possible that not enough of the food was accessible to the larvae, although they were sometimes bred in very shallow layers containing many of the heat-killed ciliates. These, like the other experiments mentioned, suggest that after the beginning of the fourth instar the food requirements of anopheline larvae are relatively great.

### DISCUSSION

It appears that this work offers a means by which the study of *Anopheles* in the larval stage is simplified; for it is necessary to deal with only one food organism, which is fairly adaptable to temperature or reaction of the medium. For adults the method here described offers the advantages which might be obtained by cultivating *Anopheles* in a sterile medium, especially as regards use of the dead or living mosquitoes for experiments on parasites, including *Plasmodia*. I have succeeded in rearing sterile adult *Anopheles*, causing them to bite a sterilized skin, and subsequently have transferred them living and sterile to test tubes, a beginning at least of a new approach to the problem of cultivation of *Plasmodia*.

### SUMMARY

A method is here described of obtaining adult *Anopheles*, living, sterile, and in workable quantities, by cultivating them in a liver extract-yeast medium in the presence of a single species of ciliate.

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# PREVALENCE 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*

The city and foreign reports which would have appeared in this issue will be published in the issue of October 27, while the State reports regularly scheduled for this issue will be published in the issue of November 3. This change is necessitated by a new arrangement with the Government Printing Office, whereby the Public Health Reports will, after a brief transition period, appear approximately on its issue date, instead of two weeks later, as at present.

## PLAGUE INFECTION IN KERN AND SAN LUIS OBISPO COUNTIES, CALIF.

Plague infection has been reported proved in a pool of 164 fleas from 35 ground squirrels, *C. beecheyi*, taken September 9 from localities east of Castaic Lake, 2 to 4 miles east of Lebec, Kern County, Calif., and in a pool of 200 fleas from 24 ground squirrels, same species, taken August 25 from a ranch 2 miles east of San Luis Obispo, San Luis Obispo County, Calif.

## COURT DECISION ON PUBLIC HEALTH

*Injunction against enforcement of milk ordinance refused.*—(Colorado Supreme Court; *Farmers' Dairy League, Inc.*, v. *City and County of Denver, et al.*, 149 P. 2d 370; decided May 22, 1944.) An ordinance of the city and county of Denver made it unlawful to sell or offer for sale within 1 mile of the exterior limits of the city and county any milk or other dairy products not produced, handled, processed, and distributed in conformity with the ordinances of the city and county and the regulations of the health department thereof when the person selling or offering for sale knew or had reason to believe that such milk or other dairy products were being purchased for use or consumption within the said city and county. A violation of the ordinance was punishable by fine or imprisonment or both. The plaintiff corporation was engaged in the dairy business and, although all of its business was carried on outside of the territorial limits of the city and county of Denver, a substantial part was conducted within 1 mile of such limits. It sought to enjoin the city and county and certain of its officers from enforcing the ordinance in question, alleging that such ordinance was unconstitutional, incapable of enforcement, and wholly void. The plaintiff further alleged that, unless restrained, the defendants, proceeding pursuant to the ordinance, would institute actions against the plaintiff to recover fines for alleged violations of the ordinance and would continue from time to time to institute proceedings, thereby creating and carrying on a multiplicity of suits against the plaintiff and compelling it to expend large sums to defend against such actions. This conduct, according to the plaintiff, would seriously impair and damage its business and irreparable injury would be done the plaintiff. The latter also averred that it had no plain, speedy, or adequate remedy at law. The defendants denied that the ordinance was unconstitutional, incapable of enforcement, or void but admitted that, unless restrained, they intended to enforce the ordinance against the plaintiff if it violated same. The defendants also answered that the complaint failed to state a claim upon which relief could be granted. The trial court gave judgment for the defendants and the plaintiff appealed to the Supreme Court of Colorado.

The latter court took the view that the plaintiff's action was not permissible, pointing out that complainants who challenged the validity of penal ordinances could urge every objection they had in defense of proceedings instituted against them. "In short, such complainants have an adequate and complete remedy at law." The

appellate court quoted from 32 C. J., p. 280, section 443, as follows: "Courts will not interfere by injunction where the injury inflicted or threatened is merely the vexation of arrest and punishment of complainant who is left free to litigate the questions of unconstitutionality of the statute or ordinance or its construction or application in making his defense at the trial or prosecution for its violation." The court did not think it fitting "that one impliedly purposing to violate a penal ordinance, as here, should enjoy predetermination at the hands of a court, which, whatever its conclusion as to the legality of the ordinance, is powerless to adjudge its enforcement."

The judgment of the lower court was affirmed.