Studies to measure the degree of inhibition by various substances of viral growth in lungs in comparison with the amount of pulmonary consolidation are being carried on.

None of the semicarbazones or thiosemicarbazones used in these studies appears to have as favorable a chemotherapeutic index as aureomycin or chloromycetin so that there is nothing to indicate their clinical usefulness in the treatment of atypical pneumonia. The observed relation of chemical structure to activity against certain viruses of large particle size including recent reports of slight activity against vaccinia in chick embryos and mice (3,4) seems of interest from the fundamental point of view and as a suggestion for further studies with other viruses in the same range of particle size.

Summary. 1. Chloramphenicol inhibited the growth of primary atypical pneumonia virus in chick embryos when injected into the yolk sac as a single dose of 5 mg one hour after amniotic inoculation of virus. Less definite inhibition of the growth of this virus in chick embryos was observed with two derivatives of 5-nitro-2-furaldehyde semicarbazone. 2. Cotton rats infected intranasally with the virus of atypical pneumonia were treated by intraperitoneal injection of several different cyclic aldehyde semicarbazone and thiosemicarbazone derivatives. Most marked suppression of the pulmonary lesions was obtained with substituted 5-nitro-2-furaldehyde semicarbazones, p-nitrobenzaldehyde semicarbazone, and p-acetylaminobenzaldehyde thiosemicarbazone.

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An Epizootic of Unknown Etiology in Guinea Pigs.* (18802)

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Several workers in diagnostic and research laboratories have observed extraneous diseases among their experimental guinea pigs. Römer (1) discovered a sporadic disease in guinea pigs which he termed infectious paralysis. The virus of this disease could be passed indefinitely by brain to brain transfer. Jackson(2) described intracellular parasites in the submaxillary glands of guinea pigs, but these were shown by Cole and Kuttner(3) to be inclusion bodies. Later Kuttner(4) found these inclusions were due to a specific salivary gland virus, and succeeded in passing the virus for 3 passages to susceptible young guinea pigs. Rosenbusch and Lucas(5) encountered a salivary gland virus of pronounced virulence while engaged in a genetics experiment; the virus was peculiar in that it was pathogenic for guinea pigs by various routes of inoculation. While conducting a nutritional experiment, Pappenheimer and Slanetz(6) were confronted with a spontaneous visceral disease in their guinea pigs. The findings were suggestive of a viral etiology, although attempted

* This investigation was aided by the Central Scientific Fund, College of Medicine, State University of Iowa.

1. Römer, *Zbl. Bakt.*, 1911, v50, 30.

2. Jackson, L., *J. Infect. Dis.*, 1920, v26, 347.

3. Cole, R., and Kuttner, A. G., *J. Exp. Med.*, 1926, v44, 855.

4. Kuttner, A. G., *J. Exp. Med.*, 1927, v46, 935.

5. Rosenbusch, C. T., and Lucas, A. M., *Am. J. Path.*, 1939, v15, 303.

6. Pappenheimer, A. M., and Slanetz, C. A., *J. Exp. Med.*, 1942, v76, 299.

passage of the agent in question yielded negative results. Van Rooyen and Rhodes(7) have described lymphocytic choriomeningitis infections in guinea pigs, and mention the possibility of wild mice being involved in the transmission of the agent. It appears likely that wild mice, or even stock experimental mice, harboring the latent infection may possibly infect susceptible guinea pigs under proper conditions. Markham(8) observed that stock guinea pigs were sometimes spontaneously infected with toxoplasmosis, and serial passage in susceptible animals was easily accomplished by brain to brain transfer.

It is the purpose of this report to describe an epizootic of 11 months duration that appeared among the guinea pigs used for tuberculosis testing by the Diagnostic Laboratory of the Bacteriology Department of the State University of Iowa in the latter part of July, 1949. This disease often interrupted the 6 weeks observation period 2 to 4 weeks prior to gross and microscopic examination of the animals for evidence of tuberculosis. All cases of the disease followed a very definite symptomatology. At the onset of symptoms the animals became unresponsive and sluggish, their coats became very shaggy and the hair shed easily, and they had a marked loss of appetite. The loss of appetite was followed by a decided loss of weight and emaciation. There was a transient increase in water consumption at this point of the disease, but soon the animals refused to drink. About this same time the guinea pigs were observed to be quite damp around the area of their chins, mouths, and ventral surface of their necks. The continual dampness of the affected pigs was perhaps the most outstanding symptom associated with the disease. In more severe cases the dampness extended posteriorly over the entire ventral surface of the abdomen. The nature of this dampness was most likely attributable to a constant nausea, accompanied by frequent vomiting, and the vomitus possessed a very acrid odor. Death of a guinea pig with such symptoms usually occurred within 6 days. When autopsied, the most significant finding was the almost complete loss of subcutaneous fat. Most of the animals showed a slight amount of hemorrhage in the meninges of the brain, and about 5% showed some lung consolidation due to a secondary pneumonia from which an alpha-prime hemolytic streptococcus was isolated.

Materials and methods. Guinea pigs used in these studies were obtained from our regular market source, and also from a different market source, and all were about 6 weeks old when inoculated. Four-week-old Swiss white mice were used from our own disease-free inbred stock. Fertile eggs were obtained from an accredited local hatchery, and the embryos were 10-11 days old when inoculated. Attempts were made to pass the etiological agent from infected to healthy guinea pigs by using various routes of inoculation, and by employing several combinations of inocula from infected animals. On one occasion, a single blind passage to healthy experimental animals from nine guinea pigs, which had been inoculated with a composite organ extract from infected animals failing to develop characteristic symptoms, was attempted. Throughout these studies complete autopsies were performed on diseased guinea pigs, and tissues were submitted to the Department of Pathology for their examination.† The possibility that we were dealing with some type of latent infection led to the inoculation of several experimental guinea pigs with materials other than organ extracts from diseased animals. The materials chosen were sterile human serum, ether extracted human serum, the lipid residue from the ether extraction, sterile milk, normal horse serum, viable B.C.G. with and without mucin, inactivated *Mycobacterium tuberculosis* with and without mucin, and gastric mucin of porcine origin.

Seven experimental animals from our regular market source and three from another source were held as uninoculated controls in the same room as the inoculated ones. Some

7. Van Rooyen, C. E., and Rhodes, A. J. Virus Diseases of Man, 1051-1060. Thomas Nelson and Sons, New York City, 1948.

8. Markham, F. S., *Am. J. Hyg.*, 1937, v26, 193.

† Appreciation and thanks are extended to Dr. Jack M. Layton of the Department of Pathology for histological examinations during these studies.

of the guinea pigs from each market source were allowed intimate contact with diseased animals, whereas others were held in separate cages as room contacts. To determine whether adult guinea pigs from our regular market source had developed an active immunity to the etiological agent in question, 10 experimental guinea pigs were inoculated intraabdominally with 1 ml of pooled adult guinea pig sera and held for observation. Attempts to transfer the causative agent to mice were made by inoculating 24 male and 32 female Swiss mice with a bacteriologically sterile composite organ extract of lung, brain, liver, spleen and spinal cord from infected experimental animals. Each mouse received 0.03 ml intracranially and intranasally and 0.3 ml intraabdominally. Using 0.2 ml of the same inoculum, 12 ten-day-old chick embryos were inoculated into the allantoic sac and onto the chorio-allantoic membrane. Penicillin and streptomycin were not added to the inoculum. The embryos were incubated at 37°C for 48 hours, after which the chorio-allantoic fluids and membranes were harvested. The chorio-allantoic fluid and supernatant fluid from the chorio-allantoic membranes from the first egg passage were subsequently inoculated into another 12 embryos as performed in the first egg passage. Thirty-six Swiss mice were inoculated with material from the harvests from both egg passages, 0.03 ml intracranially and 0.30 ml intranasally. Nine infected experimental animals, chosen from random, were

TABLE I. Results of Attempted Passage in Guinea Pigs.

No. of guinea pigs	Inoculum	Route of inoculation*	Avg days to symptoms	Avg days to death
6	brain + spinal-cord	I.C.	59	64
8	" " "	I.A.	42.5	48.5
5	brain, spleen, liver, kidney, adrenal glands	I.A.	—	—
1	liver, spleen	I.C.	62	66
1	" " , axillary node	I.C.	51	54
1	salivary gland	I.C.	50	53
Totals			44.1	47.7
22			avg	avg

* I.C.—intracranial; I.A.—intraabdominal.

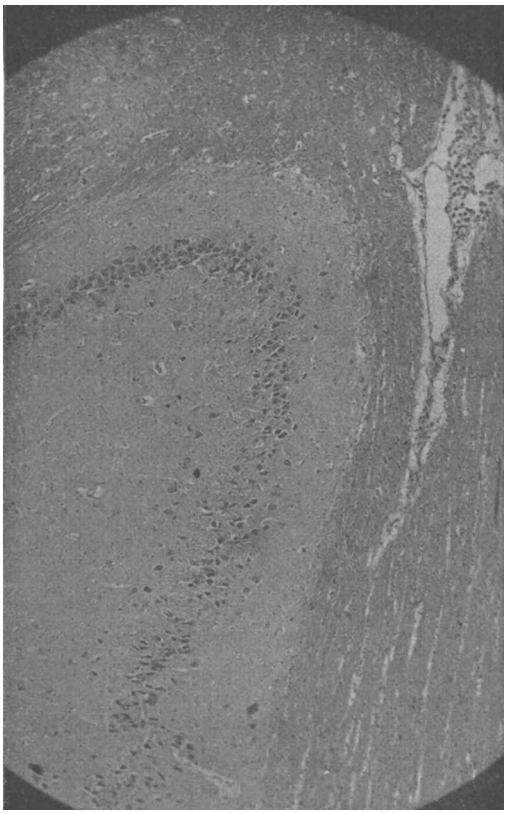

FIG. 1. Section Through Hippocampus of Diseased Guinea Pig, Low Power.

treated with four antibiotics simultaneously. Each animal received daily intramuscular injections of 0.01 g of dihydrostreptomycin, 40,000 units of S.R. penicillin, 2.5 mg of chloramphenicol, and 2.5 mg of aureomycin. Four animals were treated 4 days, and 5 for 5 days.

Results. Attempted passage from infected animals to 22 healthy experimental guinea pigs, by using the routes and various inocula shown in Table I, resulted in 17 infections. The average number of days to symptoms from date of inoculation was 44.1 and from symptoms to death 47.7 days. The one blind passage from 9 inoculated but uninfected experimental animals to healthy guinea pigs resulted in no infections.

The inoculation of 10 experimental animals in an attempt to provoke the disease with sterile milk, human serum, and other materials mentioned under methods, resulted in 8 of the 10 becoming infected in an average

time of 48.5 days from date of inoculation. At this point attention is called to the close similarity between the apparent incubation period in this experiment and the one obtained in attempted passage from infected to healthy guinea pigs.

Among the uninoculated control guinea pigs from two market sources, 8 of the 10 animals developed characteristic symptoms of the disease and succumbed. The average incubation period for those intimately exposed in both groups was 72 days, and for those held in separate cages as room contacts 85 days. No differences in susceptibility of the guinea pigs from the two sources were apparent.

The attempt to protect young healthy guinea pigs by inoculating them with pooled adult guinea pig sera resulted in failure. It was thus apparent that none of the 10 young experimental animals received passive protection from adult guinea pigs from the same stock. Attempts to transfer the etiological agent from infected guinea pigs to mice, and from infected guinea pigs to chick embryos for 2 passages and then to mice, all gave negative results. Mice failed to develop symptoms of any kind after a 6 weeks' observation period, and chick embryos failed to develop lesions and showed no toxic manifestations either on the first or second passage. Guinea pigs exhibiting typical symptoms failed to respond to treatment using four antibiotics simultaneously.

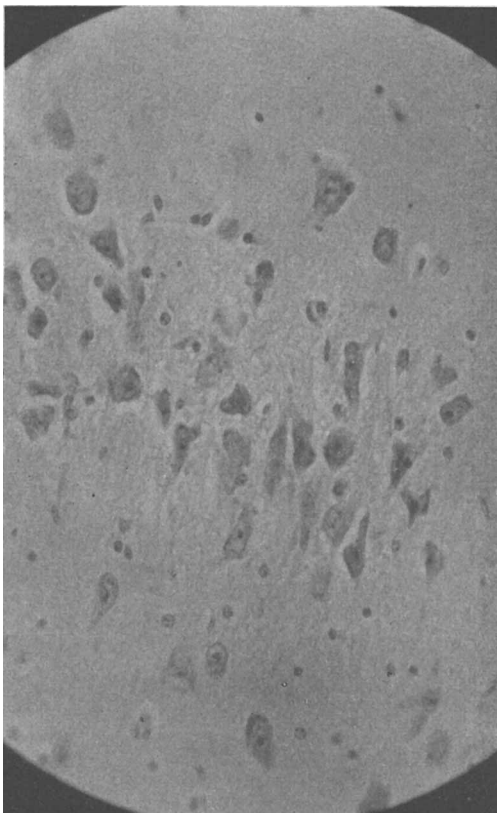

FIG. 2. Section Through Hippocampus of Diseased Guinea Pig, High Power, Showing the Degenerative Changes in the Neurones.

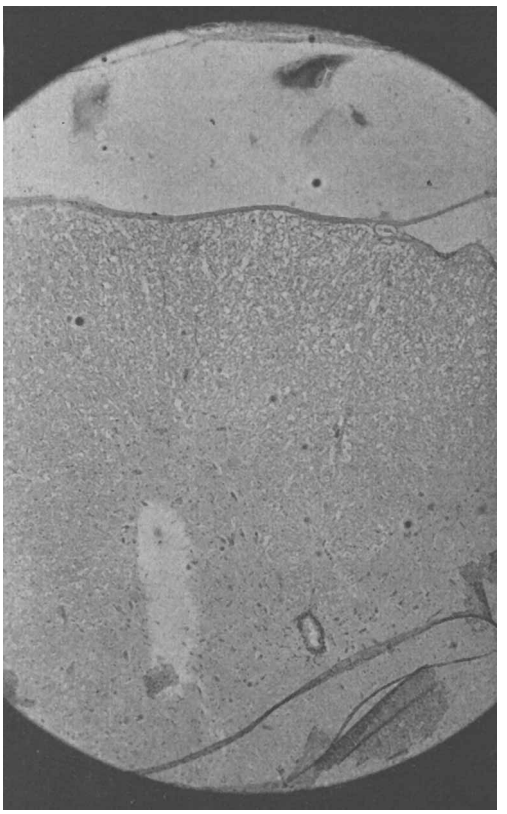

FIG. 3. Cross-Section of the Cervical Spinal Cord from a Diseased Guinea Pig, Low Power, Showing Demyelination in the Postero-Lateral Columns.

Histological sections were examined from the following tissues: Heart, lungs, thymus, lymph nodes, pancreas, skeletal muscle, gastrointestinal tract, skin, kidney, adrenal, liver, salivary glands, testicle, central nervous system, and cranium. The only suggestive ana-

tomic abnormalities encountered were in the central nervous system. Degenerative changes in the neurones of the hippocampal gyrus were constant in all of the sections from diseased animals (Fig. 1 and 2). The exact significance of the changes is difficult to evaluate. Demyelination of the postero-lateral columns of the spinal cord and patchy degeneration of neurones of the anterior horns were less constant features (Fig. 3).

Summary. Because of the inconsistency obtained in attempted passage of the etiological agent to susceptible guinea pigs, it is postulated that the etiological agent was not being passed at all. It appeared that many of the inoculations merely served to prematurely hasten an unknown etiological agent on its way, culminating in the disease pattern and the epizootic which has been described.

Conclusions. An epizootic of unknown etiology has been observed in guinea pigs. The disease was highly fatal. The symptoms and pathology were unlike any previously described disease of guinea pigs.

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A Still for the Continuous Production of Glass-Distilled Water.* (18803)

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It is frequently necessary in biological work to have a continuous supply of glass-distilled water available. The apparatus described here has proven to be entirely satisfactory for this purpose. It has been in operation in our laboratories for over a year, 24 hours a day, without a serious breakdown. During this time it has produced on the average 70 liters of glass-distilled water per day. The design is flexible and may be adapted to larger or smaller needs. The only manual labor involved in operating the still consists of filling the storage flasks and emptying the receiver flasks.

The still is shown in Fig. 1. A few features of the construction need to be emphasized. For proper operation the upper storage bottle should be tightly sealed. The condenser D on the siphon tube will condense all steam formed in this tube, thereby preventing breaking of the siphon. There should be a rapid flow of cooling water through both of the condensers. The Allihn condenser L was found to be the most efficient condenser for the purpose. The close clearance between the bulbs and the jacket produces high cooling-water velocities. The use of a Graham condenser is to be avoided due to the large back pressure produced. The vent tube on the receiver is ca 3 cm below the outlet of the condenser and leads into a drain. This is to prevent the closing of the system when the receiver is full. The outside diameter of the tubes used for the siphon and the vent system should be 8 mm. The vent on the receiver flask can either drain into the sink, or may lead to a second storage bottle. In order to prevent contamination with grease, none of the joints is greased. Glass beads or boiling stones are not required because of the even heat furnished by the heating mantle. Phosphoric acid or permanganate can be added to the boiling flask, should this be desirable. The operation of the still should be interrupted occasionally for cleaning the distilling flask. The still can be adapted to larger capacities by increasing the size of the reservoirs and the boiling flask.

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