position of the cement. The raw materials including tools are easily obtained, the cost is very small and the device is easily made. It has been used with satisfactory results in this laboratory for the past year and it is hoped that it will be of service to others in ringing slides by this method.

BASIL W. PARKER
LABORATORIES OF CRYPTOGAMIC BOTANY
HARVARD UNIVERSITY

PRESERVATION OF SMALL AMPHIBIA IN GELATIN[1]

IT is difficult to retain the natural colors and appearance of frogs and salamanders by preserving the specimen simply in formalin or alcohol. The use of tubes of gelatin, described below, overcomes this difficulty and also makes a handy mount for identification purposes in the classroom. The gelatin mixture is a clear medium through which the external features of the specimen are clearly visible. Several successive stages in the life cycle of a given species may be replaced conveniently in one tube.

Procedure: The live specimen is killed and placed at once in 20 per cent. formalin. A slit in the abdomen of the larger specimens may be made to permit more ready penetration. After remaining in the formalin over night, the specimen is removed and washed in tap water eight hours. It is next placed in Kahle's fixative over night, then washed in running tap water ten hours. Kahle's fixative is:

95 per cent. alcohol	15 parts
40 per cent. formalin	6 "
Glacial acetic acid	2 "
Distilled water	30 "

Test-tubes of from 15 to 50 cc capacity are used, depending on the size of the specimen. The mounting medium is made as follows, using Difco Standardized Bacto Gelatin:

10 grams of purified gelatin
36 drops of formalin (40 per cent.)
100 grams of water.

Heat the water to boiling, add the gelatin and allow to dissolve. Add the formalin just before pouring the mixture into the tubes. In some cases good results are obtained by adding the formalin after the gelatin has been poured into the tubes and mixing thoroughly. When the tube feels moderately warm to the hand, place the specimen in the medium in the desired position, with tweezers, etc. Further cooling will stiffen the gelatin sufficiently to hold the specimen in position. After the specimen is satisfactorily oriented, the tube may be placed in a refrigerator to hasten gelation.

The tubes are later sealed with a mixture of equal parts of Parowax and sealing wax, and should be placed in a vertical position in a rack for safe storage.

FRANK JAMES GORDON
OHIO STATE UNIVERSITY

SPECIAL ARTICLES

TRANSMISSION OF INFLUENZA BY A FILTERABLE VIRUS

THE studies of Shope[1] on swine influenza established the fact that a filterable virus is the essential factor in the production and transmission of the disease. In 1933, Smith, Andrewes and Laidlaw[2] reported that the intranasal inoculation of ferrets with nasal or pharyngeal washings from human cases of epidemic influenza produced a disease in those animals characterized by fever and catarrhal swelling of the nasal mucous membranes, but without detectable pathological lesions in the viscera. They were able to transfer the disease in ferrets by the intranasal inoculation of suspensions of the ground turbinate bones. The causative agent was found to be a filterable virus. The animals invariably recovered from the disease, and the serum of a recovered animal was found to neutralize the action of the virus. They were also able to produce a similar disease in ferrets with the virus of swine influenza. Shope[3] was able to confirm their observations on the infectivity of the virus of swine influenza for ferrets. He observed, however, that when the ferrets were inoculated intranasally under light ether anesthesia, pulmonary consolidation was an invariable accompaniment of the disease, the infection was more severe, and death of the animal sometimes occurred. Suspensions of the lungs of infected animals were found to contain a high concentration of the virus.

Last winter a number of ferrets were inoculated in this laboratory with material from various respiratory infections, including common colds, acute tonsilitis, lobar pneumonia, psittacosis and two cases of clinical influenza. In only one instance, and that distinctly of bacterial origin, was infection established in the ferret.

During the latter part of August and September of 1934, an epidemic of respiratory infection occurred in Puerto Rico. In its clinical course it appeared to be typical epidemic influenza, although the mortality was low. On September 10, 1934, through the kindness of Drs. W. C. Earle and W. A. Sawyer, of the

[1] The basis of this method was obtained from C. W. Eagleson's article in Scientific Notes, *Jour. Econ. Ent.*, Vol. 25, p. 936.

[1] Richard E. Shope, *Jour. Exp. Med.*, 54: 373, 1931.

[2] W. Smith, C. H. Andrewes and P. P. Laidlaw, *Lancet*, 2: 66, 1933.

[3] Richard E. Shope, *Jour. Exp. Med.*, 60: 49, 1934.

International Health Board, specimens of sputum in 50 per cent. glycerine, from 5 typical cases, were received. Three of the specimens were separated from the glycerine, washed and emulsified in Locke's solution. The material from each of these 3 cases was then inoculated intranasally into ferrets under ether anesthesia. On the second day after inoculation, each of the animals had an abrupt rise of temperature, which in one instance reached 106.5° F. The latter animal (P. R. 5) was sacrificed on the third day after inoculation; the lungs and turbinates were removed and ground with sand and meat infusion broth. Two animals were then inoculated intranasally with unfiltered suspensions and one with a Berkefeld V filtrate of the material. All 3 animals developed fever in from 24 to 48 hours, and, since that time, consecutive serial passages have been made in ferrets at 4 to 5 day intervals, with 5 per cent. broth suspensions of ferret lung tissue or with Berkefeld filtrates of the suspensions. In the sixth passage animal, pulmonary consolidation, bluish-red in color, was observed in the lower lobe of the left lung. In subsequent animals inoculated with either filtered or bacteria-free unfiltered material, the pulmonary lesions have been more extensive and have been consistently present through the twelfth passage. The subcutaneous injection of suspensions of lungs containing the infectious agent has elicited no evidence of the experimental disease.

In the course of the experimental work with ferrets, one of the laboratory workers (S. S.) developed symptoms typical of influenza. Nasal and pharyngeal washings inoculated intranasally induced the disease in a ferret without producing pulmonary consolidation. This strain was also transmissible from animal to animal with bacteria-free material.

The onset of fever in the animals occurs generally on the second day after inoculation. There is usually moderate apathy, lack of appetite and pallor of the nose. The catarrhal symptoms have been extremely variable. In animals with pulmonary consolidation, rapid, labored breathing and cough occur. Following the first fever, the temperature usually drops to normal and then rises again in from 24 to 48 hours. The course of the fever is not uniform. There have been no fatalities in the animals infected with the original strain which were allowed to run the entire course of the disease.

Grossly, the pulmonary involvement is usually of lobar distribution. The lung is bluish red in color. When cut, considerable moisture exudes from the tissue. The microscopical study of the involved lung of the ferret reveals a thickening of the alveolar walls with proliferation of the alveolar epithelial cells. The alveolar spaces contain, predominantly, large pink-staining cells resembling the alveolar phagocytes, many of which show degenerative changes. Polymorphonuclear leukocytes are not numerous except in the terminal bronchioles. There is also a pronounced perivascular round cell infiltration. Fibrin is scanty, but definite edema is present. The picture closely resembles the description by Shope[4] of swine influenza in ferrets. It differs strikingly from the acute bacterial infections of the lung, but is not unlike the pulmonary lesions produced by pure virus infections, such as psittacosis in the monkey.[5]

The present results confirm the observations of Smith, Andrewes and Laidlaw[6] on the transfer of a filterable, transmissible agent from human cases of epidemic influenza to ferrets. The character of the disease in the ferret differs from that described by the British authors, in that it is more severe and is accompanied by pulmonary consolidation. In these respects, the disease in our animals appears to resemble more closely the disease produced in ferrets by Shope, with swine influenza virus. There has been evidence to suggest the adaptation of the virus to the ferret, for with strain P. R. 5 distinct pulmonary lesions were first noted in the sixth passage animal.

A Berkefeld V filtrate of the sputum obtained from Puerto Rico (P. R. 5) was inoculated into 6 Swiss mice intracerebrally and intraperitoneally under ether anesthesia. Berkefeld V filtrates of suspensions of the lungs of the first and the fourth passage ferrets, administered to Swiss and white-faced mice by the intranasal, intracerebral and intraperitoneal routes, caused no demonstrable effect. In each instance the mice originally inoculated were sacrificed and passages made to new mice by the same routes.

On September 21, the centrifuged but *unfiltered* lung suspension from the second passage ferret of the contact strain (S. S.) was inoculated into 3 white-faced and 3 Swiss mice by the intracerebral, intranasal and intraperitoneal routes. One of the white-faced mice died in 24 hours with generalized bacterial infection. On the second day, several of the mice appeared to be somewhat sick, and all were killed on the fourth day. No abnormalities were found. The lungs were ground with 20 cc of Locke's solution, centrifuged, and 4 Swiss mice inoculated intranasally and intracerebrally. On the fourth day, when killed, consolidation of the upper portions of the lungs was found in 2 mice. Suspensions from these two lungs were given to 6 Swiss and 6 Rockefeller Institute mice intranasally. Several of the Swiss mice ap-

[4] *Ibid.*

[5] T. M. Rivers and G. P. Berry, *Jour. Exp. Med.*, 54: 129, 1931.

[6] *Op. cit.*

peared ill, and 4 of the 6 had definite pulmonary lesions at autopsy on the fourth day. None of the mice of the Rockefeller Institute strain showed pulmonary involvement.

In the fifth passage, death of all 6 Swiss mice and of 2 of 6 of the Rockefeller Institute strain occurred in 48 hours. The surviving mice were killed. All showed extensive pulmonary consolidation involving most of the left lung and cardiac lobe, and usually part of the right upper and middle lobes. A Berkefeld V filtrate of a 10 per cent. lung suspension was then inoculated intranasally into 6 Swiss mice. This filtrate was found to be bacteriologically sterile in both aerobic and anaerobic cultures. Death occurred in these mice in from 48 to 72 hours, all animals showing marked pulmonary consolidation.

Subsequently, the inoculation of a 5 per cent. lung suspension, or Berkefeld V filtrates of from 5 to 10 per cent. suspensions of lung, caused death in Swiss mice in about 48 hours. Mice of the Rockefeller Institute strain survived somewhat longer. In practically all instances, direct cultures of the heart's blood and of the cut lung surface have been free from bacteria. Cultures of the emulsified tissue in blood broth not infrequently reveal various Gram-negative bacilli, but they are usually few in number. These bacteria do not appear to be related to the disease process, since they are inconstantly observed. Furthermore, subsequent bacteria-free emulsions or filtrates have been fully active.

This strain of infective agent (S. S.) has been passed through 11 series of mice. A suspension of the lungs from mice of the eighth serial passage inoculated intranasally into a ferret produced a characteristic febrile reaction. At the same time, different dilutions of the mouse lung suspension were made, and a small amount of each dilution was inoculated intranasally into 4 mice. By the seventh day all animals receiving a 1:1600 dilution, or more, had succumbed with typical pathology.

More recently, an unfiltered sterile lung suspension of a ferret, the tenth passage animal of the original Puerto Rico passage strain (P. R. 5) was transferred to mice. In the first mice, killed on the fourth day, only mild pulmonary lesions were seen. In the second passage, however, all died on the fourth day with typical extensive involvement. The mice of the third passage, which received a bacteria-free 10 per cent. suspension of lung, died in 48 hours. These results are of special interest, since earlier attempts to transmit infection to mice with filtrates containing this strain were unsuccessful.

The lesions in the mouse's lung tend to involve the entire lobe, spreading peripherally. The surface is smooth and bluish gray or reddish blue. When the trachea is cut, a copious foamy liquid exudes. The cut surface of the lung is rather viscid, but firm. Stained films usually reveal mononuclear cells, but no bacteria. Microscopically, there is thickening of the alveolar walls and a moderate amount of edema in the alveolar spaces. The degree of hyperemia is variable. There is a perivascular small round cell infiltration. The cellular reaction, unlike acute bacterial infections, is predominantly of the mononuclear cells. The number of polymorphonuclear leukocytes varies. In many of the cells degenerative changes are seen in the nuclei and protoplasm.

The results of the experiments, both in ferrets and mice, indicate that the agent producing the disease in these animals is a filterable virus. It has been possible to produce the infection with filtrates, which, in aerobic and anaerobic cultures, are bacteriologically sterile. The pulmonary lesions are bacteria-free. Furthermore, the microscopic pathology of the involved lung resembles that of pulmonary lesions produced by other virus infections, rather than that of bacterial infections.

In the current issue of *The Lancet,* Andrewes, Laidlaw and Smith[7] report their success in transmitting to mice the viruses derived from both swine influenza and human influenza. The results in the present study are apparently in complete agreement with theirs. They have been able, in addition, by the use of specific antiserum from hyperimmune animals (horse and ferret), to neutralize the action of the respective viruses in mice.

THOMAS FRANCIS, JR.

HOSPITAL OF THE ROCKEFELLER INSTITUTE
NEW YORK, N. Y.

GLUTAMINE IN THE TOMATO PLANT[1]

ALTHOUGH the amide glutamine is probably widely distributed in nature,[2] very few direct attempts have been made, since its first isolation by Schulze and Bosshard,[3] to study the function of this substance in the metabolism of the plant. The lower homologue, asparagine, on the other hand, has received a great deal of attention. The hypothesis has been advanced by Prianischnikow[4] that asparagine is synthesized in the plant in response to an accumulation of ammonia from any cause. The reaction is regarded as a detoxification of the ammonia by conversion into a

[7] C. H. Andrewes, P. P. Laidlaw and W. Smith, *Lancet,* 2: 859, 1934.

[1] A part of the expense of this investigation was shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington. Dr. Clark is the holder of a National Research Council fellowship, 1933–34.

[2] A. Stieger, *Ztschr. f. physiol. Chem.,* 86: 245, 1913.

[3] E. Schulze and E. Bosshard, *Ber.,* 16: 312, 1883.

[4] D. Prianischnikow, *Biochem. Ztschr.,* 150: 407, 1924.