"RED-LEG"—AN INFECTIOUS DISEASE OF FROGS.

By HAVEN EMERSON, M.D., of New York
(Fellow of the Rockefeller Institute for Medical Research),
AND
CHARLES NORRIS, M.D., of New York.
(From the Laboratories of Physiology and Pathology at the College of Physicians and Surgeons of Columbia University.)

Having lost frogs in large numbers every autumn since 1897 from an apparently very virulent epidemic disease, we were led to investigate an unusually severe type of the trouble which destroyed two hundred of a stock of three hundred within two weeks in October, 1903.

In spite of some variations from the morphology as described by previous observers, the identity of the Bacillus hydrophilus fuscus as the essential etiological factor in the disease was definitely determined. In the course of our studies a number of observations were made both upon the pathological conditions found in the frogs, and upon the products of the bacterial cultures, which have not previously been noted. These facts, together with conclusions of practical interest, seemed sufficient reason to offer the following study in confirmation of the work of others, and in the hope that we may give assistance to laboratory workers who have suffered in a similar manner.

Previous Studies.—In an article by F. H. Russell,[7] a very complete picture of the epidemic is to be found, including the results of autopsies and the cultural findings of the Bacillus hydrophilus fuscus. Russell concludes that nothing definite can be stated regarding the mode of infection, but believes the infectious material comes to the laboratory with the frogs and gains access through superficial skin lesions, and lastly, that Chicago water does not contain the infectious agent. In addition to these observations Russell determined the presence of two toxins as

the products of pure culture of the bacillus in bouillon; one having the physiological action of digitalis, the other resembling veratrin in effect.

An organism associated with this disease of frogs was first described as Bacillus ranicida by Ernst,[2] and almost at the same time a bacillus resembling it in many respects was studied by Sanarelli,[8] who found it to be pathogenic for warm-blooded animals as well as for frogs, and named it Bacillus hydrophilus fuscus. He states definitely that his B. hydrophilus fuscus is not identical with the B. ranicida of Ernst. Both observers were led to their studies because of the loss of frogs which they were using in their studies upon anthrax. The bacillus studied by us corresponds more closely to Sanarelli's than to Ernst's.

Ernst made a few inconclusive experiments upon the effect of different temperatures upon frogs inoculated with pure cultures of the bacillus, but is inclined to believe that the frogs are able to withstand the infection better at high temperatures (20° R.) than at low temperatures (7° R.). He also notes that Rana esculenta seems to be more susceptible than Rana temporaria. He lost most of his frogs in spring and summer. Sanarelli tested the portal of entry of the infectious agent by wounding frogs with a clean scalpel and putting some in a tank with sterile water and others in the local tap water. The latter became infected, the former did not. He found the bacillus in the water supply. He concludes that the bacillus is infectious and virulent at temperatures above 30° C.; that it develops quickly in warm-blooded animals; that the filtrate from pure cultures, when injected in quantities of the same volume as were used in causing fatal infections, gave no poisonous effect. He used indefinite doses of "several drops."

Trambusti [10] also was led to his investigations because of losses among frogs in his laboratory. After identifying the bacillus and describing the clinical and pathological findings he studied (1) the physiological effect of pure cultures; (2) the physiological effect of the products of bacterial growth precipitated by alcohol; and (3) the effects of the substances soluble in alcohol.

The effect of (1) was to cause a tetanic condition of muscles and not paralysis. The effect of (2) was similar to the effect of caffein and veratrin upon muscles. The effect of (3) was paralysis.

Roger [5] observed an epidemic in June and merely notes the presence of the bacillus in question and the typical pathological findings. He also confirms Sanarelli's observations upon mammals as well as upon batrachians.

Legrain,[4] in 1888, described a gangrenous septicæmic infection among frogs, but with quite a different appearance and cause.

PRESENT STUDY.—At the time when our last epidemic was at its height, letters were addressed to a number of laboratories in this country and Canada, and, through the courtesy of the professors or their assistants in charge, twenty-one replies were received.* The name often given to the disease in these letters is "red-leg," and this is also the name used by the frog-catchers who state that the disease is well known to them and is seen very generally in the autumn months. The name is useful because it attracts attention at once to the most striking charac-

* We take this opportunity to express our gratitude for the courteous attention given by the following observers to our inquiries, whereby we obtained valuable information:

Professor C. W. Greene,	University of Missouri.
" W. P. Lombard,	" " Michigan.
" Isaac Ott,	
" Colin C. Stewart,	" " Pennsylvania.
" Arthur P. Brubaker,	
" James W. Warren,	Bryn Mawr College, Pa.
" Lafayette B. Mendel,	Yale University.
" W. R. Coe,	
" Wesley Mills,	McGill University.
" Gaylord P. Clark,	University of Syracuse.
" Winfield S. Hall,	Northwestern University Medical School.
" Theobald Smith,	Harvard Medical School.
Dr. W. B. Cannon,	" " "
Professor Graham Lusk,	Univ. and Bellevue Hosp. Medical College.
" George P. Dreyer,	
" Sidney P. Budgett,	Washington University, St. Louis, Mo.
" Theo. Hough,	Massachusetts Institute of Technology.
" George T. Kemp,	University of Illinois.
Dr. Elias P. Lyon,	" " Chicago.

teristic of the diseased frogs, the circulatory congestion of the belly and legs, varying from a faint flush to deep hæmorrhagic injection. The answers may best be arranged under the five questions asked in the circular letter:

I. Have you ever suffered from an epidemic disease among frogs?

Epidemics have been noticed, with characteristic lesions and clinical pictures sufficiently resembling those noted in our epidemic to warrant belief that they were of the same nature in and about Philadelphia and Bryn Mawr, Pa.; Cambridge, Mass.; Ann Arbor, Michigan; Chicago; Baltimore; Brooklyn; Syracuse; Montreal, Canada; Middletown, Conn.; Palo Alto, Cal.; and Columbia, Mo.

A few replies received from observers who had supplies from the same regions as those noted above, especially from Philadelphia and Chicago, state that they have never lost frogs from epidemic disease at any time of year, but this difference in the facts noted by adjacent observers may be accounted for possibly, first by the fact that these observers have had occasion to keep frogs in confinement but a short time, i. e., a week or two, during which time the manifestations of the epidemic are rarely seen. A second explanation seems to be found in the observation of certain precautions by these observers such as care in catching and cleaning and in protecting the frogs when caught from injury or infection or high temperatures.

II. What were the lesions noted, if any?

There was a general agreement as to certain features of the clinical picture and the character of the particular lesions most readily noticed.

Skin lesions, ecchymoses, and ulceration, not merely of the nose from abrasion on the side of the tanks, but of the belly and legs, were noticed by twelve observers. Three others noted ulcers on the feet and nose, with progressive sloughing of the tissues, but none of the characteristics of the epidemic under consideration. Œdematous condition before death among almost all frogs dying with the ulcers and ecchymoses was noticed by five. By two observers, evidences of convulsive muscular action were seen slightly preceding, or at the time of, death.

III. At what time of year have epidemics been seen?

Ten observers state they have seen the epidemics in the warm weather of the autumn, three noting it also in the spring. One noted July and August to be the worst months, most of the others suffering losses chiefly in September and October.

IV. Were any etiological factors noted?

The following are some of the points noted which seemed to different observers to share in causing epidemics: injury during catching, exposure to high temperatures during shipment or in the laboratories, lack of food supply, crowded conditions in the tanks, dirty tanks, tanks with linings of iron, zinc, rough rocks, or with gravel bottoms.

The clinical picture observed in our epidemic was very definite

and uniform throughout the months of October, November, and December; the variations which at first seemed essential differences were later found to be indications of the varying severity of the disease in different individuals or in groups of frogs.

The first thing about the diseased frogs which attracts attention is their sluggishness, which is often so great that they will not move to avoid a hand in the tank. They huddle together instead of jumping away. Chemical and mechanical irritation elicits but slight response. This persists and increases until there is complete failure to respond to stimuli, although if the thorax be opened the heart will be found still beating. On making kymographic tracing with the sciatic-gastrocnemius preparation the curves resemble fatigue curves, in their diminished height, prolonged relaxation period, and increased latent period. This phenomenon was so marked that it was difficult to demonstrate to a student class fatigue by comparison with fresh muscle, so immediately did the muscle fail to respond to stimuli. Distension of the lymph sacs with serum gives them a sodden and bloated appearance, making the sluggishness seem due to weight as well as to paralysis. Dulness of the coat is striking in Rana tigrina and R. viridis, the markings being less distinct, while the colors are paler than in healthy frogs under the same conditions. Congestion of all the ventral surfaces of the body from jaw to tip of hind legs is usual to greater or less degree. The head and trunk are at a much more acute angle with the basis, this position being exaggerated until just before death, when the lower jaw touches the ground and all the legs are sprawling. The attitude is such as to cause the frog to breathe under water for some time before death if there be water only to the depth of half an inch in the tank.

In several instances muscular spasms or tetanic convulsions were noted before death.

The clinical picture given by Russell [7] is similar, but he speaks of muscular spasms before death as appearing frequently, while we noticed them in only about one per cent of all deaths. The results of our autopsies were also similar to those of Russell.[7] Seventy-five frogs of one hundred and fifty which died within

three to five days of their arrival from the marshes of the Delaware and Schuylkill rivers on October 10, 1903, were examined. The lesions were remarkably uniform, but as the epidemic diminished upon the advent of colder weather, we found all grades of severity of lesions, and of suddenness of onset and duration of illness. The skin lesions consisted of hæmorrhagic papules and occasional vesicles, thirty-three per cent of the frogs showing especially large hæmorrhagic exudates under the skin at the joint of the upper and lower extremities, these latter appearing always in the frogs in which other lesions were most severe. This latter class of cases showed no recoveries, death always following rapidly after the appearance of the periarticular hæmorrhages. The extent of the hæmorrhagic skin lesions might well be taken as a measure of the severity of the disease. A few showing diffuse or petechial hæmorrhages into the skin from the mandible to the toes, varying from a slight flush to deep injection, and general hæmorrhages with ulcers, recovered; while a larger number, with a small area of congestion and a few vesicles seemed none the worse for their sickness a couple of weeks later.

The dorsal and ventral lymph spaces were distended with colorless or blood-tinged fluid, according to the severity of the attack, giving the bloated appearance so often noted, and to be explained by the grave changes in the circulating blood. In some of the frogs autopsied within an hour or two after death the heart-blood was found almost colorless and to contain very few red cells.

The tongue was found occasionally flecked with hæmorrhages. Muscular, intermuscular, and periosteal hæmorrhages were often extensive in the hind legs, accompanied by a softening of muscular tissue.

The lungs were the seat of no apparent change, but were in most instances invaded by either the Distomum cylindraceum, or by a small ascarid in large numbers, the former sometimes being encysted in the wall of the lung.*

* This is probably the Rhabdomena (Ascaris) nigrovenosum described by Miss Sheldon[9] in *The Cambridge Natural History*. We have found no other description of a parasite similar to the one seen by us found in the lungs of

When the ascarids were put into a very weak saline solution, they at once became violently contorted and gave out into the fluid countless eggs, through the thin shells of which the embryos could be seen twisting and turning. A still better view of the embryos was had by putting them in glycerine, when the shell previously cloudy though translucent, cleared.

The heart was sometimes pale, but oftener appeared to be the healthiest organ in the animal.

The stomach was uniformly distended with viscid, ropy mucus, and was markedly congested. The intestines were normal except for congestion which was frequently noted, and occasional prolapse of the anal end of the gut. The spleen and liver were soft and mottled.

Specimens taken from the lymph spaces and abdominal cavity of three or four gave uniformly pure cultures.

Essentially the same lesions were found, though in varying degrees of severity in frogs (R. viridis) previously healthy and coming from New Rochelle, N. Y., and (R. tigrina) Chicago, Ill., infected by living in the same tanks with the diseased frogs. The larger frogs succumbed much less readily than the small ones, and the large and vigorous R. tigrina from Chicago proved much more resistant than other varieties under the same conditions, and showed no deaths within a month after their receipt.

In November we secured a lot of healthy R. esculenta and R. tigrina from Ithaca, N. Y., and of these none developed the disease except on inoculation.

During October, November, and December, 1903, our frogs were received as follows:

October—300 from Philadelphia, of which 200 died of the disease. 70 were used before death, and 30 lived without showing any lesions. These consisted chiefly of R. esculenta, among which were a few of the species R. viridis.

November—25 from New Rochelle; a few of these died, but only one with lesions resembling those described above. All were R. viridis.

November—50 from Chicago, of which 10 died, although put in cleaned

---

frogs, but we have not seen the free sexually mature form of the alternating generation, as mentioned in the above description.

tanks, the rest being used for our other studies. All were R. tigrina.

December—50 from Chicago, of which 6 died of the disease, the others being used for inoculation and other studies. All were R. tigrina.

December—45 from Ithaca, N. Y., of which none died except those inoculated with the organisms of the disease. They consisted of R. esculenta and small R. tigrina in about equal numbers.

As the frogs were received they were put in soapstone sinks, each lot separate from the others, and each with an independent supply pipe and exit for water. The city water was used altogether and allowed to trickle from the sink faucet through the galvanized iron wire gauze covering the top of the sink. The sinks were occasionally scrubbed with soap and flushed out with boiling water. The water usually ran cool, but often the overheating of steam pipes raised the temperature to 35° C. None of the frogs was fed at any time.

INOCULATION EXPERIMENTS.—Three sets of inoculation experiments were made, first (A) to test the specific character of the bacillus; second (B), to see if temperature had any effect upon the course of the disease; and third (C), having found very striking effects from cold, to test the extent of the effect of keeping inoculated frogs in cold storage. The results follow.

Series A.—Four frogs (R. tigrina) from Chicago, apparently healthy, were inoculated with one-thirtieth of a twenty-four-hour agar culture of the Bacillus hydrophilus fuscus, obtained in pure culture from an early case of the disease and kept at room temperature. One was killed at the end of twenty-four hours because it presented the typical appearance of œdema, dullness, red-leg, and superficial ulceration. Autopsy showed the usual pathological appearances in the viscera and lymph. Smears of the blood showed advanced degenerative changes in the red cells, and in a few of the cells single and occasionally double bacilli were found; a few bacilli were also found extracellular. Blood cultures showed pure growth of the specific bacterium. Two others which died in forty-eight hours presented the same picture as the one described above, but the blood changes were more marked. The fourth died in fifteen days without œdema, but

with degenerative changes in the viscera and marked blood changes. This animal had thirty-eight ascarids in the lungs. It also showed the specific bacterium in the blood from the spleen but less abundantly than the others.

Series B.—Five frogs (R. esculenta) from Ithaca were inoculated on December 27th with the same strength of emulsion as was used in series A. Two were kept at room temperature and three were put in a cold-storage closet, where the temperature was never over 5° C., and always went down to 0° C. at night. The two at room temperature died within eighteen hours, and on autopsy showed great œdema and visceral degeneration, and the following blood changes: The lymph of the ventral sac was abundant and pink; no red blood flowed anywhere on incision; on opening the heart no red blood appeared; on examining a fresh smear from the heart-blood, diligent search failed to show more than one or two red cells in a field; smears stained with Jenner's stain or fixed in methyl alcohol, and stained with methyl azure and eosin, showed abundant leucocytes and many more stained nuclei of red cells, these being apparently all that were left to represent the red-blood cells.

The other three frogs were kept in the cold until January 16th, and at no time did they show the slightest sign or symptom of disease. They were alive and active on January 30th, having been kept at room temperature since January 16th.

Series C.—Twenty-four frogs were inoculated on December 29th with five minims each of a twenty-hour agar-agar slant culture at 37° C., suspended in 5 cc. of normal saline solution. The injections were all made into the dorsal lymph sac. In other words, approximately one-thirtieth of an agar culture was used. The twenty-four frogs were selected as follows: twelve large frogs of species R. tigrina (class A) from Chicago, among which there had been no disease for two weeks previously. Six small frogs of species R. tigrina (class B) and six of species R. esculenta (class C), both sets from Ithaca, N. Y., among which there had been no disease since their receipt at the laboratory. Four of the twenty-four (two from class A and one from each of the other two classes) were kept at room temperature as controls.

The other twenty were kept in the cold-storage closet under the conditions above described.

At the end of twelve hours the four controls were still alive, though dull and swollen. At the end of twenty-two hours three were dead, one of class A and the two of classes B and C. The other of class A died in twenty-seven hours. Except in the case of the last frog, which showed milder lesions, the findings were those described in the severe cases above cited.

The skin lesions were of the hæmorrhagic and serous character, but there were muscular hæmorrhages also. The blood was almost colorless, and only a few scattered red-blood cells were found. Bacilli were numerous in the lymph and blood. There were two or three of the ascarids and distoma in the lungs of each of these frogs. Of the two removed from the ice-box after a sojourn of four days, both were in perfect health, but they unfortunately escaped from the jars and died, one two days, the other six days, later, apparently as a result of drying upon the floor, as lesions of the disease were absent and no bacilli were found in smears from the lymph and blood.

Of the twenty frogs kept at low temperatures (0°–5° C.), some were allowed to remain continuously in the ice-box, and of these none died at any time.

The others were removed from the ice-box after a sojourn varying from twelve hours to thirteen days, and were then kept at room temperature. The results of this experiment follow:

Those removed from the ice-box after a sojourn of twelve hours died twenty-four hours to three and one-half days later.

Those removed from the ice-box after a sojourn of twenty-four hours died twenty-four hours to three days later.

Those removed from the ice-box after a sojourn of thirty-six hours died thirty-six hours to three and one-half days later.

Those removed from the ice-box after a sojourn of forty-eight hours died two and one-half to four and one-half days later.

Of these the one that died four and one-half days later escaped from the jar and was found dried and shrunken upon the floor, entirely lacking any of the general appearances or lesions seen in the others which died of the disease.

Of those removed from the ice-box after a sojourn of three days, one died seven days later, the other showed no appearance of disease and lived for several weeks.

Of those removed from the ice-box after a sojourn of five days, one died nine days later and one never showed any signs of the disease.

Of those removed from the ice-box after a sojourn of seven days one died nine days later, the other never showed any signs of disease.

Those removed from the ice-box after nine, eleven, and thirteen days showed no signs of disease at any time thereafter.

In other words, of the entire twenty subjected to low temperatures, after inoculation, the nine removed from the ice-box after a sojourn of four days or less showed seven deaths with lesions of the disease, one death without lesions, and one recovery.

The eleven removed from the ice-box after a sojourn of four days or longer showed two deaths, with lesions and bacilli in the blood or lymph, two deaths from accidental exposure and drying, seven recoveries. The last seven showed no signs of disease, and were alive and well thirty-two days after inoculation, although kept at room temperature continuously since removal from the cold. Of those which died, all removed from the cold under three days showed lesions of greater or less severity, and the bacilli were found present in all but one. One frog recovered after a sojourn of only three days in the cold, and the three placed in the cold chamber for only twelve hours lived longer by twelve hours to two weeks than any of those left entirely at room temperature.

The controls (i. e., the four inoculated frogs kept at room temperature) all died, while of the twenty inoculated frogs subjected to low temperatures 50 per cent recovered or died accidentally without the lesions of the disease. Of the deaths among these twenty, 60 per cent occurred in frogs which had been kept in the cold thirty-six hours or less, the proportions of recoveries increasing with the length of time they were kept in the cold.

The water in the jars in which the frogs were kept in the ice-box was always partly frozen during the night, the frogs' heads usually being embedded in the surface film of ice in the morning. The ice melted during the day as the temperature of the chamber rose (i. e., from 0°–5° C.), because of the frequent opening of the doors in daytime.

An observation, which was frequently made in the animal house in which the frogs were usually kept, was that if by acci-

dent or intention the steam heat was allowed to warm the building to 20° C., or if the water running over the frogs in their sink ran warm (30° C.) for a day because of over-heating of adjacent steam-pipes, the spread of the disease was very rapid and fatal among the frogs which had previously shown but occasional cases at intervals of several days.

If the room was warm enough to be healthy for rabbits and guinea-pigs, it was too warm for infected frogs. The nearer the temperatures of the room and water were kept to freezing the healthier were the frogs.

An opportunity was afforded in September and October, 1904, to test on a large scale the efficiency of cold in the treatment of "red-leg." Six hundred frogs of species R. tigrina were received from Chicago on September 1st, and were kept in soapstone sinks at the laboratory temperature. For ten days they appeared healthy, and only occasional deaths from injuries or accidents occurred. Suddenly they began to die in large numbers, as many as 55 being found dead in one day. The gross appearances were identical with those described in previous epidemics, and a bacillus in pure culture isolated from the lymph and the blood proved to be Bacillus hydrophilus fuscus. The average daily loss by death for the week before we made our test was twenty-six frogs.

On September 25th 304 frogs remained, of which number 144 were put in a zinc-lined tray, 2 x 3 feet, in a cold chamber, with temperature varying from 1° C. to 6° C.

On September 26th one death occurred, due to freezing, as the frog was embedded in the ice, but this animal showed absolutely no lesions and inoculations from the blood and the lymph gave sterile plates. Of this lot no others died, the tray being left meanwhile in the ice-box until October 13th; while among the 160 frogs left in soapstone sinks at laboratory temperature, the deaths continued at the rate of ten a day for three days, when 124 of the remaining healthy ones were put in the ice-box. The next day one of these was found dead and showed the typical lesions, but after that there were no further deaths.

During the week following on one occasion the temperature of the cold chamber rose to from 6° C. to 9° C. and remained at

that point for twenty-four hours, and the following morning three frogs in each of the two lots were found with marked lesions. The six were removed to separate vessels, where they lived from one to two weeks. In other words, for the week of September 19th to 25th, when about 500 frogs were kept at room temperature, our loss was 182, and for the week of September 28th to October 5th, when all our frogs (268) were in the ice-box, our loss was one from red-leg, and six already diseased frogs which lived one to two weeks.

Observations on the Blood.—Two further observations were made to control our work: one concerning the presence of the protozoan Drepanidium in the red-blood cells, the other with reference to the anæmia and leucocytosis present in the frogs dying from the disease.

Several of the frogs received in October, 1903, from Philadelphia which were examined showed in stained smears of the blood, in addition to the abundant typical bacillus, a marked leucocytosis and the presence of a hæmatozoan parasite in the red cells. Comparison with the slides and drawings of Dr. Langmann [3] given in his paper on the subject showed the organism to be the Drepanidium he described.

Subsequent search for this organism, to test its association with some of the lesions or characteristics of the disease, showed that it was present in only two per cent of the Philadelphia frogs and in one per cent of the frogs from other sources. There was no reason to consider it in any way related etiologically to the disease in question. This is in accord with the observations of Dr. Langmann, who found this hæmatozoan parasite to be without effect upon the life history of snakes.

In regard to anæmia among the frogs, two distinct causes were found for the severe grade present in a number, one of which may properly be classed as a predisposing factor in some cases, and the other as a direct and constant result of the bacterial infection.

The normal red-cell count in Rana temporaria, according to Rollett [6] and Bethe,[1] is 393,200 per cubic millimetre. In a number of apparently healthy frogs of different kinds the red-

cell count was found by us to vary from 354,000 to 500,000 per cubic millimetre. In a number of frogs with poor musculature and dull skin, but without any lesions of "red-leg" or any leucocytosis, the count varied from 114,000 to 354,000 per cubic millimetre.

In these frogs we found many distomes in the lungs. These parasites live upon the red-blood cells of the frog, which they suck from the pulmonary capillaries. On spreading a distome out upon a slide and compressing it slightly with a cover-glass, the intestine can be seen loaded with the oval nucleated red cells of the frog. Estimating the length of the canal by use of a micrometre scale, following the contortions of the tube, and then counting the number of red cells in a given straight length of the tube, the number of red cells in a single parasite was found to be 300,000. As these parasites were found in some frogs to the number of twenty and over, a total of at least 6,000,000 red cells may be estimated as having been drawn from a frog at a given time.

The total blood bulk of the frogs varied from 0.75 cc. to 1.5 cc. Taking 500,000 red cells per cubic millimetre as a basis of calculation, we have an estimated total number of red cells of 375,000,000 to 750,000,000 per frog, of which 1.6 per cent to 0.8 per cent would be at one time in the digestive canal of the parasites, whose very active digestion makes the process of erythrocytic destruction rapid and progressive.

These parasites were found in healthy frogs, and they were absent in many of the diseased frogs. Thus their presence is not a constant factor, but it seems probable that the anæmia they cause might diminish the resistance to infection or prevent recovery when infection has occurred.

The presence of the ascarid even in numbers up to twenty-six in a single animal appeared to have no relation to any morbid state of the frog. In many instances we found both kinds of parasites in one or both lungs of the same frog, whatever the source of the supply, but the occurrence of the distome was most common in the first supply of Philadelphia frogs which we received.

The constant occurrence of marked and severe blood changes

in the frogs dying of "red-leg" has already been noted, and it needs merely to be repeated that in the severer infections a careful search is required to discover a single red corpuscle in fresh and stained smear preparations of the blood. This observation led us to make a few experiments upon the effects of the filtrate from broth culture grown for a week at 37° C. The filtrate had no effect upon the red corpuscles; but a very different picture was presented by a hanging-drop preparation of a 1-10 saline dilution of normal frog's blood, and an emulsion of a twenty-four-hour agar-agar culture of the bacillus. From being distributed evenly through the drop, the very motile bacilli were, in the course of two hours, seen in clustered masses about the red cells. In three to four hours the cells showed irregular outlines and appeared paler. In ten to twelve hours nothing appeared to represent the cells but the nuclei about which were the now sluggish groups of previously actively motile bacilli. The three facts so often noticed at autopsy, namely, the severe laking of the blood, the presence of numerous isolated red-cell nuclei, and the almost total absence of red corpuscles in the severer cases, find a plausible explanation in the destruction of the red cells, as observed in the hanging-drop preparation.

It must be noted, however, that the great excess of bacilli in proportion to the red-blood cells in the hanging-drop preparation is in marked contrast to the actual conditions observed in the blood of infected frogs. And further, it should be explained that even though the filtrate has no effect *in vitro* upon the red corpuscles of the frog, it must be granted that there is a possibility that the products of the growth of the bacilli in the body may differ from those produced under artificial conditions and tested upon red-blood cells in a test tube.

MORPHOLOGY AND BIOLOGY OF BACILLUS HYDROPHILUS FUSCUS.—The bacillus isolated from the frogs affected with the so-called "red-leg" has striking biological characters. The following is a description of the morphology, and of the pathogenic and biochemical properties, of the bacillus.

Morphology.—As is the case with many bacilli, the morphology varies considerably, according to the source from which it has

been obtained. The tinctorial properties of the organisms likewise vary, depending upon the stains employed for their demonstration.

Thus in the blood of the frog the bacillus is a fairly thick rod, exhibiting, by Jenner's blood stain, marked bi-polar and other irregularities of staining. A form commonly seen in the fresh blood smears is a shovel-shaped bacillus. The square ends of these forms appear strikingly fringed, and it is by these ends that they often appear in apposition as diplobacilli. With the ordinary dyes, however, the bacterial plasma is uniformly colored. The rods appear single or as diplobacilli, and they are seen within both the white and red corpuscles, in the former being present in considerable numbers, especially in frogs about to die. Just before death, and in the blood of dead frogs, the bacilli may be present in large numbers.

In young cultures on all media shorter and longer rods predominate, while coccoid forms and diplobacilli are also present. The bacillus may be said to be somewhat thicker than the typhoid bacillus, the rods being straight, and on the average the length is five to six times the breadth. In older agar cultures—after four days to several weeks—and on potato the rods remain short and thick, although filaments and forms staining poorly are met with occasionally in abundance. After several days to a week's growth on coagulated ox serum, the organism becomes markedly filamentous and long rods and bizarre involution forms are frequent.

Young cultures stain intensely with all the ordinary dyes. No spores are developed. The bacillus is very actively motile, and does not retain Gram's stain. The thermal death point was not ascertained.

Pathogenicity.—The marked pathogenic action of the cultures on frogs has been described above. On guinea-pigs emulsion of young agar cultures exerts a toxic and rapidly fatal action, one-tenth of a twenty-four-hour culture introduced into the peritoneum or subcutaneously producing death within twelve hours. The site of inoculation in the subcutaneous tissue is the seat of hæmorrhagic infiltration. Guinea-pigs inoculated in the peritoneum

develop a marked peritonitis with blood-tinged exudate. Cultures taken from the heart's blood of guinea-pigs just after death develop a few colonies. The fatal action is thus toxic and septicæmic.

Rabbits, on the contrary, are not susceptible. One-tenth of a 24-hour agar culture produces a slight and transient indisposition, when inoculated by way of the ear vein; and if by way of the skin small abscesses which tend to heal.

The pathogenic action on other animals was not determined.

Cultural Characteristics.—The growth is rapid and abundant upon the usual media, the optimum temperature being about 37° C., although growth is abundant at room temperature and at temperatures as low as 12° C.

The colonies upon agar and gelatin present no diagnostic characters.

The plates on 12 % gelatin, "2 % acid to phenolphthalein," develop colonies which are yellowish by transmitted light, and irregularly marked. When small and young, they are pale and slightly stippled. Liquefaction is complete in two days in the cold room, and well-isolated large colonies cause advanced liquefaction within two days.

The superficial colonies on agar are whitish, somewhat raised and moist, and are coarsely stippled. The circumferential portions of the colony are pale and have even borders, the central portion is decidedly yellowish and is marked by round aggregations. The deep colonies are round or oblong, deep yellow in color, and irregularly lobulated.

Young cultures are odorless. Old cultures, however, acquire a rank odor.

Gelatin Stab.—A small globular-shaped area of liquefaction develops beneath the surface of a gelatin stab culture. The liquefaction increases slowly in extent and is not complete for several weeks. A whitish pellicle is soon formed and a heavy, whitish deposit settles out promptly. No greenish tint was observed. Gelatin stabs grown for two days and then melted remain fluid at room temperature. After three days' growth the gelatin remains fluid when placed in cold storage.

Milk.—Milk is completely clotted in twenty-four hours at 37° C. Liquefaction of the clot is apparent within four days, and is practically complete in ten days. At room temperature clotting and liquefaction occur more slowly than at incubator temperature.

The rapidity and character of these processes vary somewhat in different cultures.

In litmus milk, "1.5 % acid to phenolphthalein," a marked acid reaction promptly develops. The coagulum, instead of being solid, may be finely divided, and then settles out promptly, leaving the fluid clear. The clot never completely disappears, even after four weeks, and may or may not retain its acid color.

In milk rendered alkaline (0.2 cc. $\frac{N}{1}$NaOH to 10 cc. milk) the reaction becomes acid, and a finely divided clot appears in two days at 37° C., which is soon liquefied, the fluid becoming clear. In milk rendered acid (0.2 cc. $\frac{N}{1}$ HCl and 10 cc. milk), the same succession of changes is observed, as also in similar sets of milk-tubes grown at room temperature, the reaction in the latter case taking place more slowly.

The Enzymic Action of Filtrates of Broth Cultures upon Milk and Gelatin.—The sterile filtrates of broth cultures in milk bring about changes similar to those caused by the living bacillus.

The following tests were made:

Litmus milk 10 cc. + 1 cc. broth filtrate (1.3 % acid; culture grown twenty-eight days at 37° C.). Soft clot third day at 37° C., fifth day clot settles out and liquefaction proceeds slowly. After four weeks a small clot sticks to the sides of the tube, the fluid being left perfectly clear.

In litmus milk, to which 0.2 cc. $\frac{N}{1}$ NaOH was added, decolorization begins promptly, coagulation occurs at two days, clarification is complete on the fifth day. The control milk-tube, to which no filtrate had been added, showed the same changes, due to alkaline clarification of the milk, after the fifth day.

In milk rendered strongly acid by addition of 0.2 cc. HCl, clotting and liquefaction of the clot occur as in the milk without addition of acid.

In milk-tubes kept at room temperature clotting is delayed until the tenth day, and the liquefaction is much less extensive and complete than in the tubes kept at 37° C.

The peptonizing action of filtrates on gelatin is rapid and complete.

1 cc. filtrate (growth of four weeks) added to 6 cc. of melted gelatin (12 %) produces permanent liquefaction in two days, 0.5 cc., in five days, while 0.1 cc. requires several weeks to produce slight softening of the gelatin.

The liquefying action of the filtrate (1 cc.), when added on top of a column of gelatin, is much slower, one inch of gelatin being rendered fluid in a week, the action being complete after four to five weeks.

The peptonizing action of the filtrate is greatly accelerated in the incubator.

The filtrates of young broth cultures are apparently more active than those obtained from older cultures.

The filtrate of a three-day broth culture grown at 37° C., reaction 1.75 acid to phenolphthalein gave the following action upon milk:

5 cc. filtrate added to 10 cc. milk, twenty-four hours at 37° C., produced a marked clarification of the milk, in three days the fluid becoming perfectly clear, a small coagulum being left at the bottom of the tube.

1 cc. of the filtrate added to 10 cc. milk causes a solid clot in twenty-four hours, which becomes peptonized in a couple of days. The enzymic action of the filtrate at room temperature is considerably slower than at incubator temperature.

Gelatin, 5–7 cc., to which when fluid 1 cc. of the filtrate is added, remains permanently fluid at room temperature.

Broth.—Marked turbidity occurs within twenty-four hours, and flocculi and a heavy pellicle soon develop.

Potato.—On potato (natural reaction 0.5 % acid to phenolphthalein) at room and incubator temperature the growth is abundant, wet, slightly raised, and yellowish. This yellow color changes in the course of a few days to a reddish hue, assuming later a rusty-colored sputum tint.

In four weeks the potato is covered by a heavy and dull brownish-colored growth, the upper and dry portion of the growth presenting a greenish, metallic lustre.

On the same potatoes rendered alkaline by 0.2 % NaOH, the growth never turns reddish in color, but is abundant, the early citron color turning slowly into a brownish gray.

On acidified potatoes (HCl) the growth is scanty and invisible.

The pigment production on potatoes was found to vary considerably, the reddish tint not appearing constantly, but being much more marked in those potatoes, especially when dry, inoculated from cultures recently isolated. On dry potatoes the

growth was markedly raised and wrinkled, and of an intense reddish brown or terra-cotta color.

The red pigmentation was observed only on the potato.

Slant Agar.—The whitish, moist streak spreads rapidly, and the growth assumes a yellowish tint in the course of time. The yellow pigment production is more marked in the early than in the later subcultures. The yellowish pigmentation of the early cultures became intense when grown for several weeks.

Coagulated Ox Serum.—Abundant whitish growth; no pigment formation; gradual softening of the serum, the liquefaction in the tubes kept at 37° C. being concealed by the evaporation.

Fermentative Action upon Sugars.—When freshly isolated from frogs and tested upon various sugars (Theobald Smith's sugar-free broth with addition of 1 % of sugar), the bacillus fermented mannite, dextrose, and saccharose with the production of acid and gas. The gas production, however, was slight, a large bubble of gas developing in the course of several days at 37° C. Both arms of the fermentation tube became markedly turbid.

Lactose was never fermented with gas production, although the growth in both arms of the fermentation tube was abundant.

After cultivation for a few generations upon agar, the bacillus lost its property of fermenting sugars with gas production. The property of growing in the closed arm of sugar-free broth in Smith's fermentation tube, as shown by the turbidity after twenty-four hours' growth at 37° C., was retained, but in a lesser degree.

The growth in the open arm of the tube containing sugar-free broth and lactose broth is abundant, a heavy, whitish deposit settling out in a few days.

The growth in the open arm of the tubes containing dextrose, mannite, and saccharose broth is not nearly as abundant as in the lactose and sugar-free broth, the cloudiness in both arms being practically similar.

An examination of the following table indicates that the most probable explanation of the slight growth in the open arm is found in the production of acid in the fermentation of the sugars

(dextrose, saccharose, and mannite), which acid inhibits further growth.

TABLE TO SHOW THE AMOUNT OF ACID PRODUCED IN SUGAR-FREE BROTH (1 % ACID TO PHENOLPHTHALEIN), TO WHICH 1 % OF VARIOUS SUGARS HAS BEEN ADDED.

Grown 24 hrs. at 37° C.	*7 days (idem.)*	*4 weeks (idem.)*
Open arm, 1 %	1.1 %*	Not tested.
Closed arm, 1.3 %	2 %	" "
1 % Lactose		
Open arm, 1.1 %	1 %*	Not tested
Closed arm, 1.3 %	2.2 %	2.3 %?
1 % Dextrose		
Open arm, 2.2 %	2.7 %	4.1 %
Closed arm, 2 %	1.8 %	3.2 %
1 % Saccharose		
Open arm, 2.3 %	2.8 %	4.4 %
Closed arm, 1.9 %	2.2 %	3.1 %
1 % Mannite		
Open arm, 2.1 %	2.5 %	Not tested
Closed arm, 1.1 %	2.2 %	" "

Fermentation Reactions in Hiss's Serum Media.—† Hiss's, 1 % dextrose-, mannite-, saccharose-, maltose-, dextrin-and-starch-, litmus serum water (1-3) becomes clotted firmly in twenty-four hours at 37° C.

The clot in the dextrose tubes remains acid and solid for weeks. Softening of the clot begins in the other sugar serum waters after three days, but never becomes liquefied to any extent, except in the dextrine tube. The clot is an acid clot.

In 1 % lactose and 1 % inulin-litmus serum water (1-3), the color, as in the plain sugar-free litmus serum water, remains unchanged, no acid reaction being developed. The serum water becomes opaque, and finally solid in four days. Softening slowly takes place, the clot being almost liquefied in three weeks, and the litmus is decolorized. The clot in these tubes is a "sweet" clot due to a rennet-like ferment.

* Rose tint on adding phenolphthalein.

† Hiss, *Centralb. f. Bakt.*, 1902, xxxi, 302; *Science*, 1902, March 7, p. 367; *Medical News*, New York, 1903, February 14, p. 6.

The filtrates of broth cultures have no action upon the various sugar serum water media, thus 1 cc. of a 28-day filtrate leaves the serum (5 cc.) unchanged after several weeks at 37° C.

From these observations the conclusion seems justified that the fermentation of the sugars is produced through the action of an intracellular bacterial ferment, that is, one which is not extracted or set free, or at least not in sufficient quantities, during the growth of the bacillus in the broth cultures, and is thus absent in the filtrates. In other words, the fermentation of the sugars with acid production is induced only through immediate contact of the living bacillus with the sugar, the filtrates leaving the reaction of the litmus unchanged, as seen in serum water tubes.

In marked contrast to these ferments are those soluble ones we have already described, which peptonize gelatin, and which coagulate and subsequently liquefy or peptonize milk and serum.

We do not know definitely whether the rennet-like ferment and the peptonizing ferment are distinct. The bacillus does not split urea or asparagin with acid production, as 1 % urea and asparagin litmus peptone solutions are unaffected.

Reduction of Nitrates. Indol.—The bacillus rapidly reduces nitrates to nitrites, and indol is slowly produced in Dunham's peptone solution.

An intense color reaction is developed in the nitrate peptone solution in which the bacillus has been grown for twenty-four hours at 37° C., when tested with the sulphanilic or "naphthalymine test." After five days' growth the nitrites are completely reduced, no color reaction being now obtainable.

Growth for twenty-four hours in Dunham's peptone solution develops a marked reddish color on addition of concentrated sulphuric acid, while in tubes grown for two days no color reaction develops. After four days' growth at 37° C. a faint rose tint develops on addition of sulphuric acid and the nitrite solution. The color reaction becomes intense, when the tubes have been grown for from two to four weeks. The deep cherry-red color then obtained varies somewhat from the usual tint obtained with the indol test.

THE IMMUNE BODIES DEVELOPED BY ADAPTATION OF RABBITS TO THE BACILLUS.—Agglutinins and precipitins are developed in rabbits adapted to emulsions of living bacilli, as well as those inoculated with filtrates of broth cultures. Thus in a rabbit inoculated with living organisms an agglutination limit of 1-12,500 in two hours ("microscopical clumps") was obtained, the precipitation limit being slightly above 1-10 (28-day filtrate).

We were unable to demonstrate the presence of anti-ferments in the immune sera in the few tests that were made. It was found that normal as well as immune sera have a marked inhibiting action upon the enzymes of the filtrates, but an accurate study of the differences between the normal and the immune serum was not made. One-tenth of one cubic centimetre of both normal and immune serum prevents the coagulation of 10 cubic centimetres of milk by 1 cubic centimetre of filtrate, and the peptonization of gelatin by 1 cubic centimetre of filtrate.

CONSIDERATION OF SPECIES OF BACILLUS ISOLATED.—We have not succeeded in positively identifying the bacillus. It, however, resembles in most of its cultural characteristics the bacillus described by Sanarelli, the Bacillus hydrophilus fuscus, and also corresponds to Russell's description of this bacillus.

Our bacillus corresponds more closely to Sanarelli's bacillus than to Ernst's Bacillus ranicida. It does not develop pigment on agar, except for a yellowish tint which appears in older cultures in varying intensity, and thus differs from the bacillus as described by Sanarelli and also by Russell. Both of these observers state that their bacillus is pathogenic for rabbits; our organism, however, has only slight pathogenic properties upon these animals.

The variations noted above are, however, insufficient to warrant us in defining a new species and for the following reasons: the bacillus when first isolated by us produced a small amount of gas, whereas later subcultures fermented sugars without gas production. Similarly the pigment production in the early subcultures was more marked than in the later subcultures. The pigment formation, indeed, was so variable that no constant factors controlling its occurrence were determinable.

When first isolated the cultures of 1904 corresponded closely to the initial cultures taken in the fall of 1903, in respect to the pigment formation on potato, the marked enzymic action upon gelatin, milk, and coagulated serum, and the fermentation of sugars and gas production. Unfortunately, we have not been able to determine the loss of gas formation in later cultures of the bacillus of the epidemic of 1904, as was found to be the case in the previous year.

We have not been able as yet to determine, by means of the reaction of agglutination, the group to which our bacillus belongs. A strong anti-serum for Bacillus prodigiosus (1-60,000) fails to agglutinate our bacillus in any dilution, so that we may possibly be justified in excluding it from this group. In determining the group and species characters of a given micro-organism, it is, however, questionable how far cultural features and agglutinability should be taken into account. Thus among the prodigiosus group, races which closely resemble each other in cultural characteristics do not possess similar agglutinative affinities, and an anti-serum developed for one species fails to agglutinate other species which closely resemble the first.

Conclusions.

The epidemics we have observed were due to the presence and growth in the frogs of Bacillus hydrophilus fuscus. This was proved by recovering the bacillus in pure culture from the body fluids of frogs sick or dead of the disease, and the inoculation of healthy frogs with an emulsion of the pure culture, and by obtaining the same clinical picture and pathological findings as in the original diseased frogs; and, finally, by recovering the bacillus in pure culture from frogs inoculated and sick or dying as a result of the inoculation.

The disease is widely distributed throughout North America and Europe, and in this country and Canada is known as "red-leg."

It has been observed by us chiefly in the warm weather of September and October.

The disease is characterized by congestion of the ventral surfaces of the body, with more or less ulceration in, and hæmorrhage beneath, the skin, bloating due to serous exudation into the lymph sacs, gradual failure to respond to stimuli, which symptoms are followed by coma and death, the last being occasionally preceded by tetanic seizures.

After death hæmorrhages into the muscles and degenerative changes in the muscles, spleen, liver, and, to a slight degree, in the intestinal tract, are found. The blood shows an advanced degree of anæmia and leucocytosis.

Predisposing causes of the disease are lesions of the skin, which seem to be the usual portal of entry of the infection, and lowered resistance from heat and from anæmia.

By a series of controlled experiments with inoculated frogs we have shown that, while temperatures a little above freezing have no harmful effect upon the frogs, they completely control all manifestations of the disease in inoculated or diseased frogs, if the frogs are left in the cold for a period as long as seven days; and, further, that even short periods in the cold chamber will bring about a delay of the fatal results in diseased or inoculated frogs.

The anæmia so often found in apparently healthy frogs seems in many cases to be due to the presence in the lungs of the frog of a parasite, the Distomum cylindraceum, which, occurring in sufficiently large numbers in an individual frog, is capable of materially diminishing the available supply of red corpuscles.

Severe laking of the blood, the presence of numerous isolated red-cell nuclei, and great diminution in the number, or almost total absence of the red cells in the diseased frogs, are in proportion to the severity of the infection and due to bacterial action.

The presence of the hæmatozoan parasite, the Drepanidium, does not play any part as a predisposing or exciting cause of the disease.

The ascarid Rhabdomena nigrovenosum, although frequently present as a parasite in the lungs of the frogs, plays no part in causing or promoting the disease.

Precautions to be Observed in Preventing or Checking Epidemics in Laboratories.

If frogs are to be kept for more than a few days, care should be taken to avoid supplies from those marshes where the disease is known to prevail year after year, hence the Delaware and Schuylkill valleys which show the disease in an endemic form had best be avoided.

Frogs should not be caught by barrel-staving or any other violent method which may cause abrasions of the skin. The use of a soft, fine-meshed hand-net is to be preferred to other methods.

Before shipment all injured or very pale or thin frogs, or any showing a suspicion of redness of belly or legs, should be put aside, and the rest washed for twenty-four hours in a full stream of cold water.

Shipment should be made in planed wooden boxes, free from projecting knots, nails, or rough surfaces inside. In warm weather ice in some metal vessel placed in the box is to be recommended for delaying over-heating in transit for short distances.

When received, the frogs should be washed thoroughly in an abundance of clean cold water, and should then be kept in a cellar where the temperature in winter is a little above freezing and in summer about 15° C.

The ideal tank is one with smooth sides and bottom and supplied with fresh cold water. The following description of a tank sent to us by Professor George T. Kemp, of the University of Illinois, is so complete that we take the liberty of quoting from his letter:

"The tank consists of an earthenware sink, 3 x 2 feet and 6 inches deep, lined with a hard glaze. It stands on a frame giving it such a tilt that one-third of the bottom is dry when there is 2 inches of water at the lower end. There is a hole in the lower end fitted with a cork, through which runs a glass tube $\frac{3}{8}$ of an inch in diameter, extending into the sink 2 inches from the bottom. The tank is covered by an ordinary window-sash, in which one pane is replaced by a galvanized wire gauze. Water

trickles into the sink from a pipe opening above the gauze and runs out through the glass tube in the cork in the sink-hole. The frogs can shift from wet to dry as they please. The whole sink can be easily disinfected by using sodium hypochlorite with a scrubbing brush. One hundred frogs can be kept in such a tank."

Treatment of the disease when it occurs may be summed up in the words cleanliness, water, and cold.

If the disease appears among frogs in a tank, those with any lesions, such as ulcerations or hemorrhages, or even faint flushing of the belly or legs, should be put into a separate cleaned tank and kept as near 0° C. as possible.

The rest should be removed from their tank to a clean one after being freely washed with an abundance of cold water; the infected tank should be first scrubbed with soap and water, then with a 1-20 carbolic acid solution, and finally flushed out with fresh water. Prof. Colin C. Stewart, of Dartmouth College, uses inclined tanks in series, one water supply sufficing for all the tanks, the diseased frogs being put in the lowest tank as soon as they have been discovered in any of the upper ones. He finds that this method of weeding out the sick ones, together with one thorough cleansing of all the tanks, will check an epidemic, although those already infected may die.

The use of low temperature will in our experience save almost all of those already diseased.

BIBLIOGRAPHY.

1. Bethe, M.—*Schwalbe; Morph. Arb.*, I, 207–240.
2. Ernst, P.—*Beiträge zur pathologische Anatomie und allgemeine Pathologie*, 1890, viii, 203.
3. Langmann, G.—*N. Y. Medical Journal*, 1899, lxix, 1.
4. Legrain, M. E.—*Revue médicale de l'Est*, 1888, xx, No. 11.
5. Roger, M.—*Comptes rendus de la Société de biologie*, 1893, 709.
6. Rollett, A.—*Stricker; Handb. Lehre von den Geweben*, 1091 and 1141.
7. Russell, F. H.—*Journal Am. Med. Assoc.*, 1898, xxx, 1442.
8. Sanarelli, G.—*Centralblatt für Bakteriologie und Parasitenkunde*, 1891, ix, 193, 222.
9. Sheldon, L.—*Camb. Nat. Hist.*, vol. ii, p. 140.
10. Trambusti—*Beiträge zur pathologische Anatomie und allgemeine Pathologie*, 1893, xiv, 317.