STUDIES IN BACTERIOSIS.

I. "BLACKLEG" OF THE POTATO.

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INTRODUCTION.

THE losses caused by bacteriosis of the potato in this country are fortunately not so great as they appear to be in other parts of Europe, the United States, and in Canada. Compared with fungal diseases those of bacterial origin are quite of secondary importance and do not usually cause any serious trouble. In most districts a certain number of plants succumb each year to attacks of bacteria but they are so few that very little notice is taken of them, they are usually pulled up and burnt, but sometimes are left standing in the field without detriment to the neighbouring plants. The chief losses are incurred during storage in the *clamp* or *pie*, and unless special precautions to insure a good system of ventilation are taken in building the clamp the conditions of warmth and moisture may become so favourable to the growth of bacteria that a few diseased tubers included in the crop act as foci from which disease spreads. In this way the whole, or a large part, of the store occasionally becomes involved. If on any field a more than usual number of plants showing signs of bacterial disease are noticed during the summer the wise man takes the precaution not to store the crop from this field but to send it at once to market.

Epidemics are of such rare occurrence in this country that the subject has received but little attention by English pathologists. In Canada however the losses from this cause have assumed much more serious proportions, Harrison (6) found the average percentage of rotted tubers during three years as high as 22 per cent., and the report of the crop correspondent of the Canadian Bureau of Industries for 1905 shows losses from rot ranging from 10 per cent. to 75 per cent. In Galicia also the percentage of diseased plants was very high during 1907—1910; von Hegyi (8) reported that 40 per cent. to 60 per cent. of the crop was attacked in some experiments in which "seed" from a badly infected area was used. Although the extent of loss of potatoes by bacterial disease is at present low, probably not more than 5 per cent. of the entire crop of Great Britain, it may at any time become much more serious, and in fact there are indications that it is steadily on the increase. The Board of Agriculture (21) recognises two bacterial diseases of the potato namely *Blackleg* and *Brown Rot*; these have been identified by the outward symptoms and the presence of bacteria in the tissues, but the actual isolation and identification of the causal organisms has not been attempted. It seems therefore desirable that a thorough investigation of these diseases should be carried out and the present paper embodies the results of a study of the former disease as it occurs in Lancashire.

SYMPTOMS OF THE DISEASE.

The symptoms of the disease known as "Blackleg" have been described very fully by Appel (1) and more recently by Pethybridge and Murphy (15) so that it is unnecessary to give more than a brief description of the general characters of the disease. It usually makes its appearance early in the summer during June or July, and especially when a spell of hot weather follows a rainy period. On looking across an infected patch of potatoes one sees here and there a plant distinguished from the rest by the wilted and yellow appearance of its leaves; these later become dark brown or almost black and very much shrivelled. On close inspection the stem shows at the ground level a blackened area which gradually spreads upwards as the disease progresses. Such stems when pulled gently leave the soil with scarcely any resistance, there being as a rule an entire absence of development of tubers. The pith at the base of such stems is completely rotted away leaving a hollow space surrounded by a more or less healthy cortex in which the vascular bundles stand out very prominently in virtue of a strong brown pigment in the walls of the vessels. If the attack has occurred late in the summer the disease will have spread from the main stem through the underground stems to the developing young tubers, and if these are cut longitudinally through the "heel" they show the vascular ring marked out by the same brown pigment which stains the vascular bundles of the stem. In many cases this browning is limited to the bundles at the "heel" end only; this is usually the only sign of disease exhibited in the young tuber, the storage tissue having quite a normal appearance. Microscopic examination of a section across one of these bundles shows the vessels filled with a mass of bacteria: from the vascular bundles the organism under suitable conditions rapidly invades the starchy tissue reducing it to a soft pulpy mass with very offensive smell. Potatoes in such a condition are frequently found in the field, especially if rain be followed by a hot baking sun. In the important potato-growing districts of Lancashire these tubers, thus rotted in the soil, are locally termed "*par-boiled.*" Microscopic examination of the pulpy mass reveals the cells separated from one another, the substance of the middle lamella being apparently dissolved by an enzyme; the starch grains appear to be quite unaltered.

The propagation of the disease is usually assumed to be due to the planting of diseased "sets"; von Hegyi (8) however who investigated "Blackleg" in Galicia and Prussian Silesia has stated that in every case the "blacklegged" shoots examined by him bore evidence of the attack of *wire-worms*, and that biting insects are a necessary factor for the entry of the parasite into the host. The fact that the parent "set" from which the diseased shoots have arisen will almost invariably be found to have decayed points to the planting of diseased "sets" as the source of the disease in most cases. This is further emphasised by the fact that the disease makes its appearance in isolated individuals and is only rarely to be found affecting even small patches. The general lack of anything in the nature of an epidemic of "Blackleg" spreading from a certain focus of infection would seem to indicate that propagation by wire-worms is not usual, though undoubtedly biting insects may be instrumental under certain conditions in introducing the parasite from the soil. Morse (14) gives one instance of such an epidemic spreading over a patch of potatoes where the soil was exceptionally moist, and it is probable that insects were the responsible agents in this case.

ETIOLOGY.

The disease known as "Blackleg" appears to be caused by a number of different parasites. *B. phytophthorus* was isolated and named in 1903 by O. Appel (1) in Germany and the same organism has been found by E. F. Smith (18) and L. R. Jones (10) as causing the disease in America. In 1911 Pethybridge and Murphy (15) described the disease under the name of "Black Stalk Rot." The organism which they isolated from diseased plants in Ireland differs only slightly from that described by Appel, and they suggest that it is probably only a variety of the same organism, but since it differs constantly in certain particulars they rather reluctantly formed a new species *B. melanogenes*. The disease investigated by F. C. Harrison (6) in Ontario resembles in outward characteristics that of "Blackleg," as described by Appel, except that while in the latter the woody bundles remain hard and strong enough to support the diseased shoot so that these are conspicuous objects standing erect in the field, in the former the vascular bundles are so softened that the wilted and blackened shoots fall to the ground and lie hidden so that on casual inspection the crop has the appearance of being perfectly healthy.

Mention must be made here also of a rotting disease of the potato ascribed by E. F. Smith (19) to *B. solanacearum* since the final wilted appearance of the shoots might be mistaken for the symptom of "Blackleg." In this disease however the progression of the wilt is from above downwards, the infection occurring in the leaves through the agency of leaf-biting insects, while in "Blackleg" the lower leaves are the first to show signs of wilting since the point of infection is subterranean. Besides these a number of other organisms have from time to time been described as producing rot in the potato accompanied by the appearance of "blacklegged" shoots. Thus we find *B. atrosepticus* described by van Hall (5), *Micrococcus phytophthorus* by Frank (3), *B. caulivorus* by Prillieux and Delacroix (16) and *B. solanincola* by Delacroix (2).

Of these *Micrococcus phytophthorus* was possibly not the cause of the disease which Frank had before him. Various workers have stated that Micrococci appear abundantly on plate cultures from diseased stems but that these prove to be saprophytic organisms. Frank describes a special resistance to rotting exhibited by the potato in winter. He states that his cultures during September and October produced rapid rotting while those of December and January had no pathogenic properties. While it is quite conceivable that physiological changes in the tuber might result in such a resistance it seems more probable, as Appel suggests (1), in view of the frequent occurrence of saprophytic Micrococci, that Frank was dealing with mixed cultures probably containing *B. phytophthorus* in which a saprophytic Micrococcus predominated, and that by December when the "special resistance" made its appearance the real parasite had been lost. *B. caulivorus* was considered by Laurent (12) and Griffon (4) to be identical with *B. fluorescens liquefaciens*. Both hold that under certain conditions of the soil common saprophytes may take on the characteristics of parasites and produce disease in plants. Laurent holds an alkaline condition of the soil to be the essential factor, while Griffon believes that atmospheric humidity, moist soil and a variety of potato of low resistance are the necessary factors which induce rotting by *B. fluorescens*. Neither Laurent nor Griffon appears to have made convincing infection experiments with *B. fluorescens*, the latter in fact states that his attempts in this direction were not encouraging, and as Riehm points out in an abstract of Griffon's paper (17) when the author shows the presence of this organism in plants which are decomposed he has no proof of the pathogenicity of the bacillus. The question whether bacilli of the fluorescent type are capable of producing disease in the potato is thus in some considerable doubt. Jensen (9) brought evidence to show that many ammonia-producing organisms may enter living plants through wounds in virtue of the lethal effect of ammonia upon the cells, so that dead material continually presents itself to the invading organism.

Doubt has also been raised by E. F. Smith (20) as to the pathogenicity of the organism described by Delacroix under the name *B. solanincola*. A culture of this organism obtained by Smith was not virulent, and an examination by him of the material left by Delacroix as typical of the disease revealed no bacteria in the vessels; on the other hand there was considerable evidence that fungal parasites had been the cause of the disease. Delacroix's description of the disease seems to show that it had a bacterial origin and it would be unsafe to place too much weight upon the evidence to the contrary which Smith's research has produced, since the material to which he had access might not have been quite typical of that examined by Delacroix and the culture might have lost the virulence which it had once possessed.

We have then four or five different organisms which have been described in other countries as giving rise to the symptoms of "Blackleg" but up to the present no one has fully identified the cause of the disease in Great Britain, hence earlier statements by Massee (13) and others to the effect that it is due to *B. phytophthorus* are not supported by the evidence at present available. This work was undertaken with the purpose of determining which of the several organisms are present in this country. Since this paper was written a publication by Morse (22) has come to hand in which it is shown that three of these organisms, namely *B. atrosepticus*, *B. solanisaprus* and *B. melanogenes*, when cultivated under the same conditions give identical physiological reactions and possess morphological characters which differ so slightly that, on these alone, a separation into distinct species is not warranted. These must therefore be considered as strains of one species to which Morse applies, on grounds of priority, the name *B. atrosepticus*.

COLLECTION OF THE MATERIAL.

During the months of summer when "Blackleg" makes its appearance the weather in 1916 was particularly dry over most parts of England so there was perhaps less of this disease than is normally the case. However when this investigation was begun in the second week in August it was reported that there had been rather a considerable amount of it in certain parts of Wiltshire and Devonshire and also in parts of Surrey and Kent. The material investigated was collected during a survey of Wart Disease in the Ormskirk District of Lancashire. Stalks showing the symptoms of "Blackleg" were not numerous, in fact on many fields they were difficult to find. This was mainly due to the fact that the weather had been particularly dry for some weeks. Several diseased stalks were pulled up here and there and cultures were made the same night by inoculating from the most recently diseased portions of the pith upon slopes of potato-mush-agar. On the day of leaving the district a plot some twelve drills wide and of about two acres area was found in which the percentage of "blacklegged" stalks was very high, about one plant in six showing the disease. This plot had no pegs or other marks to distinguish it from the neighbouring plots but the appearance of "Blackleg" upon it was so marked that its limits could be most distinctly seen on looking down the drills. Search was made on a neighbouring plot but not a single case of a "blacklegged" shoot could be found. This was the more extraordinary since the farmer gave the assurance that the seed was the same (King Edward) and from the same source, that the manurial treatment had been the same (namely a dressing of Fison's Mixed Artificial), moreover the soil type appeared to be identical on the two plots. The only difference lay in the setting of the "seed"; in the one case use had been made of a home-made dibbling machine while in the other the drill had been ploughed and the "seed" set in the ordinary way. In the former case presumably the "seed" had been set nearer the surface than in the latter and this would seem to have given rise to conditions favourable to the action of bacteria. As this suggests a possible method of control in bacterial diseases experiments will be made during this summer in order to test the efficacy of deep planting in checking "Blackleg." Some of the tubers from the diseased plot in question have been obtained for this purpose.

ISOLATION OF THE ORGANISM CAUSING THE DISEASE.

In view of the fact that the material was collected rather late in the year the separation of the parasite from the great number of accompanying saprophytes has proved very difficult and tedious. Many attempts at isolation by the method of "poured plates" have given only disappointing results; five out of thirty of the original cultures of mixed organisms produced on potato slices vigorous rotting which could be carried on from potato to potato, but whenever attempts were made to obtain the active organism on plates of bouillon-gelatine or bouillon-agar the colonies that developed invariably proved to be those of organisms which had no power to produce rotting[1].

Successful isolation was finally obtained as follows: from one of the original cultures on potato-mush-agar a water suspension of the mixture was made and the cut end of a shoot of potato was immersed in it. After four days the stem showed characteristic blackening, and rotting of the pith. This rotten pith was then inserted in a hole made by a half centimetre cork-borer in a potato tuber which had been sterilised with mercuric chloride. After two days incubation at air temperature rotting was well established and from the margin of the rotted area a loopful was taken and inoculated upon potato-mush-agar. Attempts at isolation of the parasite from this culture by "poured plates" again proved abortive. A water suspension was made from the growth on potato-mush-agar and the cut end of a bean shoot (*Vicia faba*) was immersed in it, there being at this time no shoots of potato available. In four days the pith was well rotted to a height of five centimetres from the end. Poured plates in alkaline bouillon-agar were then made from the pith at the limit of disease. Several colonies appeared after three days and these were tested in turn as to their rotting power on a potato slice. Some thirty colonies were tried in this way with negative results, but three weeks after plating one colony was discovered which proved to be of a pathogenic kind. Exactly when this colony appeared it is impossible to say as it was quite old when tested and had ceased to grow. This proved to be a vigorous

[1] Many of these colonies were produced by Micrococci and the regularity of their appearance suggests that they represent the organism described by Frank under the name *Micrococcus phytophthorus* and this should be considered a *nomen nudum* until a pathogenic form answering to Frank's description shall be re-discovered.

organism producing lively rotting of the potato. Whereas the impure cultures had always given from the first a pinkish-brown colour in the rotted tissue or a mixture of brown, black and yellow, the rot produced by the pure culture was not coloured at all until about a week after inoculation, when a slight pink tinge developed in the rotted part bordered by a brown stain in the surrounding tissue which finally turned almost jet black.

After two transfers to fresh sterile potato slices the organism was plated out on bouillon-gelatine and incubated at 19° C. On the second day thirteen colonies all apparently similar had made their appearance and had produced a basin-shaped liquefaction of the gelatine with a diameter of four to five millimetres. Each of the thirteen colonies was inoculated upon potato tissue and gave rise to the rot characteristic of the organism as described above. This was taken as proof of the purity of the culture and examination of the organism in stained preparations and under "dark-ground" illumination further verified this.

DESCRIPTION OF THE ORGANISM.

I. MORPHOLOGICAL CHARACTERS.

Form and Size. The organism is a short rod with rounded ends. When taken from the diseased tissue after 48 hours incubation at 20° C. the length of the single rod varies from $1{\cdot}2\mu$ to $2{\cdot}4\mu$ and the breadth from $0{\cdot}7\mu$ to $0{\cdot}8\mu$; pairs of organisms are very numerous and measure up to $3{\cdot}5\mu$ in length. Taken from bouillon-agar the length of the rods is more variable and pairs of organisms are not of such frequent occurrence but the organism has a tendency to form in chains. The measurements were made upon preparations fixed five minutes in 4 per cent. formalin, stained 10 minutes in aqueous methyl violet and examined in oil.

Motility and Flagella. The organism is an actively motile bacillus and swims with a rapid rotation on its long axis. The flagella are peritrichous and three to six in number when taken from an agar slope, but many are uni-flagellate when taken directly from diseased tissue. Beautiful stained preparations of the flagella were obtained by a modification[1] of van Ermengen's method and also by Löwit's method.

[1] This was a modification by Stevens and I am indebted to Dr R. S. Williams for having introduced it to my notice. Details of the method are given in Hewlett's *Bacteriology*, 5th Edition, 1914, page 115.

Staining. The organism stains well with the usual bacteriological stains. It exhibits no polar or other characteristic granules. It does not stain by Gram's method. Spores have not been observed.

II. Cultural Characters.

Stab Culture in Bouillon-gelatine. In acid gelatine (titrating + 10 of Fuller's scale), after 24 hrs. at 20° C., growth was visible to the bottom of the tube and liquefaction had commenced at the upper part of the stab. After 48 hrs. liquefaction was "infundibuliform" and had spread to about 8 mm. diameter at the surface. A granular deposit formed in the liquid gelatine and gradually sank to the bottom. The reaction of the liquid was strongly alkaline to litmus. In alkaline gelatine (– 1·5) growth was similar but rather more rapid.

Stab Culture in Bouillon-agar. Growth was granular and uniform throughout the length of the tube. The organism is obviously a facultative anaerobe. No difference in growth could be observed on media of acid or alkaline reaction.

Streak Culture on Bouillon-agar. After 24 hrs. at 20° C. the streak was 1·5 to 2 mm. wide with smooth margin; dirty white by reflected light; raised; "wet-shining." When collected in a mass on a platinum wire a faint yellow colour was perceptible.

Plate Culture on Bouillon-gelatine. After 48 hrs. at 20° C. the colonies had made their appearance and those on the surface had liquefied the gelatine; liquefaction basin-shaped; bluish-grey turbidity; granular deposit at bottom of depression.

Plate Culture on Bouillon-agar. Colonies visible after 48 hrs. at 20° C. Surface colonies dirty white; brown by transmitted light; round; "wet-shining"; raised ½ to 1 mm. above the surface; domed. Deep-lying colonies "punctiform."

Streak Culture on Potato-mush-agar. After 24 hrs. at 20° C. streak about 2 mm. wide and of a light chrome-yellow colour.

Streak Culture on cooked Potato. After 48 hrs. at 20° C. strong growth and chrome-yellow colour, surrounding tissue of a mouse-grey colour.

Bouillon + 5. Bouillon turbid after 24 hrs. at 20° C. No pellicle formed at the surface and no ring where the liquid meniscus came in contact with the glass. After four days a considerable deposit had formed at the bottom of the tube.

Potato Broth. Became turbid after 24 hrs. at 20° C. On the second day there was in one case a slight sign of a pellicle but this broke up on shaking, sank to the bottom and did not re-form. In three other tubes there was not the slightest sign of pellicle formation. After eight days the liquid was neutral to litmus, and a small bubble of gas had collected in a Durham's tube.

III. Physiological Characters.

The culture used for these experiments was prepared as recommended by Harding and Morse(7) in their report of investigations on the physiological behaviour of various strains of soft-rot-producing organisms, successive transfers being made from bouillon to bouillon-gelatine and then to bouillon-agar, one night's incubation at 20° C. being allowed between each. A tube of Dunham's solution was inoculated copiously from the last and a loopful of this employed for each test. In all cases unless otherwise stated the temperature of incubation was 20° C.

10 *per cent. Witte Peptone* + 1 *per cent. Glucose.* Became slightly acid on the second day; on the fourth day it had become strongly acid and a small bubble of gas had collected in a Durham's tube. After fourteen days the gas occupied about 1/20 of the Durham's tube.

10 *per cent. Witte Peptone* + 1 *per cent. Lactose.* Acid appeared on the third day and gas on the fifth day. Final volume of gas as above.

10 *per cent. Witte Peptone* + 1 *per cent. Saccharose.* Acid and gas appeared on the fifth day. Volume of gas as above.

10 *per cent. Witte Peptone* + 1 *per cent. Mannite.* Acid and gas appeared on the third day. Volume of gas was about three times as large as in the cases of the other sugars.

Uschinsky's Solution. No acid and no gas produced. The litmus showed slight alkalinity and on the fourth day commenced to bleach from the bottom of the tube upwards. After fourteen days the liquid was entirely decolorised except for a depth of a few millimetres at the surface. The colour was partially restored on shaking.

Potato Broth + 2 *per cent. Glucose.* After eight days incubation at 25° C. reaction very slightly acid (practically neutral). Three minute bubbles had collected in a Durham's tube. No trace of pellicle formation.

Potato Broth + 2 *per cent. Saccharose.* After eight days at 25° C. reaction slightly acid. Bubble of gas less than 0·1 c.c. No sign of pellicle formation.

Nitrate Bouillon. Growth wa more vigorous than in bouillon without nitrate. Nitrite was present after 24 hrs. and was still present after thirty days.

Diastatic Action. This was tested on a piece of potato standing in water. After fourteen days the liquid gave a true red colour with iodine, showing that all the starch had been hydrolysed. The diastatic action of the organism is fairly strong despite the fact that the starch grains appear to be unattacked in the cells of rotted potato tissue.

Milk. Coagulated on the sixth day. The curd was quite loose and was easily broken by gently tapping the tube. On the twentieth day the whey was well separated from the curd. The curd was still present thirty days after inoculation.

Litmus Milk. Became slightly acid on the third day, distinctly so on the fourth day; coagulation occurred on the ninth day, loose curd as above; the whey titrated on the thirtieth day was acid to the degree 0·06 N (+ 60 of Fuller's scale).

Dunham's Solution. Growth was not vigorous, only a slight turbidity was formed. When tested after fourteen days there was no sign of the presence of indol.

Thermal Death Point. 60 c.c. of potato broth was inoculated and incubated over night at 25° C. The turbid liquid was divided into six sterile tubes and these were subjected for ten minutes to temperatures ranging from 48° to 55° C. Inoculations made from these on potato-agar showed that the organism was killed at temperatures above 49° C.

INFECTION EXPERIMENTS.

Sterile Slices of living Potato. The potato was sterilised with mercuric chloride, washed three times with sterile water and cut into slices with a flamed knife. The slices were placed on wet blotting paper in sterile petri dishes. At 20° C. rotting was well established after 24 hrs., had penetrated to a depth of about 2 mm. and had spread to a radius of 1 cm. from the point of inoculation; at this temperature the whole of the tissue was decomposed. The rotted tissue had a strong alkaline reaction to litmus and gave off a volatile base which was identified by the sense of smell as trimethylamine; no other odour was perceptible. As stated earlier the rot was perfectly white and remained so in the incubator but on exposure to light a rose-pink colour developed. At air temperature 12° to 14° C. the rot usually progressed throughout the depth of the slice (5 to 6 mm.) but after spreading to a radius of 1 to 1·5 cm. from the point of infection it was frequently prevented from further advance by a resistant layer of cork; this cork formation was accompanied by a brown stain in the tissue bordering the rotted area and finally the whole of the unattacked tissue assumed a dark brown to jet-black colour.

Sterile Slices of other Vegetables. At 20° C. vigorous rotting was produced in slices of carrot, white turnip, yellow turnip, celery, onion, Jerusalem artichoke, parsnip and sugar-beet. At air temperature these all rotted to a certain extent but much less rapidly than at 20° C., and rotting was frequently checked as in the case of the potato by a protective formation of cork.

Cut Shoots of Potato and Bean (Vicia faba). Shoots of these two plants placed in tubes containing 10 c.c. of a suspension of the organism in distilled water and kept in a green-house at 54° to 57° F., commenced to rot at the cut end on the second day. The rot accompanied by characteristic blackening of the stem steadily advanced upwards, so that after eight days a length of some four or five centimetres of the stem had been completely decomposed. Controls under the same conditions remained perfectly healthy for three weeks.

Prick Infection of the Stem of growing Plants. The plants used had been forced in pots during the winter in a green-house with a regulated temperature of 54° to 57° F. When the infections were made the plants inoculated had sturdy upright stems eight to ten millimetres in diameter and twenty to twenty-five centimetres in height, but leaf production was very meagre and some of the plants were showing signs of precocious flowering. They were not very robust specimens but served quite well for the purpose of this research. When infection was to be made below the level of the soil this was removed to a depth of about two inches and subsequently replaced. Before inoculation the stems were washed with alcohol and allowed to dry, puncture infections were then made with a stout platinum wire bearing the inoculating material, and the stems were bound closely with one centimetre strips of tin foil in order to protect the wounds against the entrance of soil and loss of moisture. In all, ten plants were inoculated in this way, four below and six above the level of the soil. In every case successful infection resulted while control plants remained perfectly healthy. On three occasions the organism was successfully re-isolated from such infected stems. One typical experiment may be cited in detail. The stem of a plant bearing one shoot only, 25 cms. in height and 8 mm. in diameter, was inoculated as described one inch below the level of the soil with material from a rotted potato slice. This culture had been incubated over night at 20° C. and represented the eighth transfer from the original, and the third transfer from the isolation of the organism in a state of purity. The first sign of rotting of the stem was observed on the tenth day after inoculation; on the thirteenth day the stem was black to a height of two centimetres above the level of the soil; it was then pulled up and found to show the signs characteristic of the disease, intense blackening of the epidermis, dark brown stain in the vascular bundles and complete destruction of the pith at the base of the stem. The epidermis was seared along one side with a hot knife and cut longitudinally, the sides were laid back with sterile forceps and a portion of the most recently attacked pith was transferred to a tube of sterile water. At the same time a portion of the pith was placed upon a slice of potato and after 24 hours at 20° C. had produced a white rot some 4 mm. in depth and of 1 cm. radius. Dilution plates in bouillon-gelatine were made from the water suspension of the diseased pith and after 48 hours incubation fifty colonies had developed on the first plate, these were all similar in appearance and had produced liquefaction, as described earlier, varying from 1—3 mm. in diameter. A loopful from each of ten of these colonies was "spotted" on a slice of sterile living potato and in each case characteristic rotting of the tissue resulted, thus the four rules of Koch for the establishment of the pathogenicity of the organism were fully complied with.

COMPARISON OF THE ORGANISM WITH PREVIOUSLY DESCRIBED PRODUCERS OF "BLACKLEG."

The organism here studied differs from *B. caulivorus* or organisms of the fluorescent type in not producing a green fluorescence in artificial culture and from *B. solanacearum* and *B. solanincola* in its power to liquefy gelatine, a character which these two organisms do not possess. It is also clearly different from an organism which has been described by Kramer (11) as causing a rot of the tuber in that it does not produce gas and butyric acid when cultivated on potato tissue and in certain nutrient solutions. There remain to be considered *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes* and *B. phytophthorus*. The first three of these as stated earlier (page 484), have been shown to be strains of one and the same species by the fact that under certain cultural treatment the physiological differences upon which separation of these was based fail to appear. The description which Morse (22) gives of the characters of these organisms after they had been cultivated through several changes of potato-broth agrees almost entirely with the description given in this paper and with the original description of

Pethybridge and Murphy (15) for their species *B. melanogenes*. It is thus clear that in all these cases the same organism is being dealt with and in agreement with the opinion of Morse the proper designation of the organism is *B. atrosepticus*. Since several strains of this organism have been isolated in different localities it is of interest to note that the one found in Lancashire is the same as that previously found in Ireland.

It is to be regretted that Morse was unable to obtain a virulent culture of *B. phytophthorus* since it has been shown by Pethybridge and Murphy (15) that this species differs only very slightly from their organism, and it seems probable that a comparison of this organism along with the others studied by Morse would have proved it to be identical with them. However, being unable to obtain a trustworthy culture of *B. phytophthorus*, and in deference to the opinion of Smith (18) that this species is not identical with *B. solanisaprus*, Morse was forced to exclude it from the species *B. atrosepticus*. Since the statement by Smith was in all probability based upon physiological differences which have been shown by Morse to be inconstant a comparison of the cultural characters of *B. phytophthorus* and *B. solanisaprus* on the lines laid down by Morse is highly desirable.

SUMMARY.

The organism which produces "Blackleg" of the potato in Lancashire is *B. atrosepticus* (van Hall).

It is in all respects identical with the organism responsible for this disease in Ireland and described by Pethybridge and Murphy under the name *B. melanogenes*.

REFERENCES.

1. APPEL, O. Untersuchungen über die Schwarzbeinigkeit und die durch Bakterien hervorgerufene Knollenfäule der Kartoffel. *Arb. a. d. biol. Anst. f. Land- u. Forstwirthschaft*, Bd. III, s. 364, 1903.
2. DELACROIX, G. Sur une maladie bactérienne de la pomme de terre. *Comp. rend.*, CXXXIII, p. 417, 1901.
3. FRANK, A. B. Die Bakterienkrankheiten der Kartoffeln. *Centralbl. f. Bakt.* Abt. II, Bd. V, s. 98 u. 134, 1899.
4. GRIFFON, ED. Sur le rôle des bacilles fluorescents de Flügge en Pathologie végétale. *Comp. rend.*, CXLIX, p. 50, 1909.
5. VAN HALL, C. J. J. Bijdragen tot de Kennis der bakterieelle Plantenziekten. Dissertation. Amsterdam, 1902.
6. HARRISON, F. C. A Bacterial Rot of the Potato caused by *Bacillus solanisaprus*. *Centralbl. f. Bakt.* Abt. II, Bd. XVII, s. 34, 1907.

7. HARDING, H. A., and MORSE, W. J. The Bacterial Soft Rots of certain Vegetables. *New York Agric. Exp. Sta. Tech. Bull.*, No. 33, 1909.
8. VON HEGYI, D. Einige Beobachtungen betreffs der Schwarzbeinigkeit der Kartoffel. *Zeitschr. f. Pflanzenkr.* XX, s. 79, 1910.
9. JENSEN, H. Versuche über Bakterienkrankheiten bei Kartoffeln. *Centralbl. f. Bakt.* Abt. II, Bd. VI, s. 641, 1900.
10. JONES, L. R. *Vermont Exp. Sta. Report*, XIX, p. 257, 1906.
11. KRAMER, E. Bakteriologische Untersuchungen über die Nassfäule der Kartoffelknollen. *Oesterreich. landw. Centralbl.* Heft I, p. 11, 1891.
12. LAURENT, E. Recherches expérimentales sur les maladies des plantes. *Annales de l'Institut Pasteur*, Tome XIII, p. 1, 1899.
13. MASSEE, G. *Diseases of Cultivated Plants and Trees*, London, 1910.
14. MORSE, W. J. "Blackleg," a Bacterial Disease of the Stem and Tuber of the Irish Potato. *Maine Agric. Exp. Sta. Bull.*, No. 174, 1909.
15. PETHYBRIDGE, G. H., and MURPHY, P. A. A Bacterial Disease of the Potato Plant in Ireland. *Proc. Roy. Ir. Acad.* XXIX, Sect. B, p. 1, 1911.
16. PRILLIEUX AND DELACROIX. La gangrène de la tige de la pomme de terre, maladie bacillaire. *Comp. rend.* CXI, p. 208, 1890.
17. RIEHM, GR. Abstract of (4) above. *Zeitschr. f. Pflanzenkr.* XX, s. 426, 1910.
18. SMITH, E. F. *Science*, XXXI, p. 748, 1910.
19. ——. Bacterial Disease of Tomato, Egg-plant and Irish Potato. *U.S. Dep. of Agric., Div. of Veg. Phys. and Path.*, Bulletin No. 12, 1896.
20. ——. Footnote on page 215, Vol. III. *Bacteria in Relation to Plant Diseases*, Washington, D.C., 1914.
21. Leaflets of the Board of Agriculture, Nos. 117 and 242.
22. MORSE, W. J. Studies upon the Blackleg Disease of the Potato, with Special Reference to the Relationship of the Causal Organisms. *Jour. Agric. Research*, VIII, p. 79, 1917.

(*Received April* 19*th*, 1917.)