

ON THE DISCOLOURED SPOTS SOMETIMES
PRESENT ON CHILLED BEEF, WITH
SPECIAL REFERENCE TO "BLACK SPOT."

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(With Plates VI and VII.)

This investigation was carried out at the Jodrell Laboratory, Kew Gardens, and formed part of an inquiry made by Dr J. M. Hamill, on behalf of the Local Government Board, England, into the occurrence of mould on imported beef.

DURING the years 1909 and 1910 Dr J. M. Hamill, Inspector of Foods, Local Government Board, submitted to the Jodrell Laboratory, Kew, for investigation, examples of refrigerated beef imported into this country from Argentina, showing on the surface blackish, or variously coloured spots, supposed to be due to the presence of fungi. This surmise proved to be correct, as in every discoloured spot a fungus was found to be present, and furthermore, when such fungi were isolated and obtained as pure cultures, similar spots were produced when carefully sterilised portions of chilled (refrigerated) beef were infected with spores obtained from the cultures.

A preliminary examination revealed the fact that only one kind of fungus, producing blackish spots, was constantly present; the remaining species, seventeen in number, fall under the popular category of "moulds," such as frequently occur on stale bread, cheese, jam, and other forms of organic matter.

Many of these fungi are of European origin, but are now met with wherever civilised man has penetrated, and their occasional presence on chilled meat must be considered as purely accidental; in other words, they have not up to the present evolved any special adaptation, that has enabled them to benefit by selecting chilled meat as a desirable nutrient substratum.

In the case of what is commonly called the “Black Spot” fungus, on the other hand, adaptation to chilled beef as a food-supply is very marked, and has reached the stage of specialisation expressed by the term “biological species,” which in the present instance implies that the fungus has so far departed from the normal type of the species that the typical form of reproduction by spores has been abandoned, and has been replaced by a secondary and lower form of reproductive bodies specially adapted to suit the new set of circumstances under which it flourishes. This form of reproduction is illustrated in Fig. 4. The very minute spores (conidia) are produced in chains on the surface of the substratum in immense numbers, and are readily dispersed by currents of air, etc. These spores are capable of germination at the moment of maturity, and constitute the primary source of infection. Immersed in the substratum a second type of reproductive bodies called chlamydo-spores are produced from specialised cells of the vegetative hyphae of the fungus. These chlamydo-spores or resting-spores germinate only after a period of rest (Fig. 5), and will even pass uninjured through the intestinal canal of a carnivorous animal (cat).

Macroscopic Characters of the Fungus causing Black Spots.

The spots occur on the subcutaneous areolar tissue, first appearing as minute scattered points, which gradually increase in size up to 1—rarely 2—cm. in diameter, and retain a more or less circular outline. The circumference is seen to be fringed with dark-coloured radiating hyphae when examined under a low-power lens. The spots when small and young are dark grey or leaden in colour, gradually changing as they become larger and the mycelium becomes more abundant and denser, to a dull black with a tinge of olive-green. As a rule the spots are separated from each other by a distance of two to six inches, or even more, as in Fig. 2, but in bad cases the spots are present in groups in close proximity to each other (Fig. 1).

Microscopic Appearance of “Black Spot” Fungus.

When a section through a mature “black spot” is examined under the microscope the substance of the subcutaneous tissue is seen to be traversed by a densely interwoven web of olive-coloured hyphae. The stoutest of these hyphae average about 8μ in diameter, are transversely septate and copiously branched; the branches are usually given off at

about a right angle to the parent axis. The youngest branches are almost colourless, and in the older branches the colouring matter is located in the cell-wall, the contents remaining perfectly colourless. When the spots are young all the hyphae are completely immersed in the substratum ; at a later stage, however, fertile hyphae appear above the surface and produce their crop of spores in the free air, for the purpose of facilitating dispersion. At the same time a few scattered hyphae grow erect into the air. These represent the conidiophores or fruit-bearing branches which in the normal condition of the fungus bear the reproductive bodies or spores, but which in the modified form of the fungus growing on chilled beef, persistently remain dwarf and sterile. The superficial spores are produced in long chains at the tips of somewhat slender hyphae, and vary in form from almost perfectly globose to broadly elliptical, the latter form being most abundant. The cell-wall is smooth and dull olive-green ; size somewhat variable, ranging between 5 and 8 μ in diameter, or 5—8 \times 4—5 μ . The chlamydo-spores, buried in the substratum, are variable in form and size, and are produced singly or in short chains, 12—20 μ diameter, or 12—24 \times 10—12 μ , wall thick, smooth, deep olive-green at maturity.

Penetration of the Fungus into the Substratum.

In no single instance has the fungus been observed to penetrate to a greater depth than 4 mm. into the substratum. As a rule it is confined to subcutaneous connective tissue and to the fascia enveloping the muscles, and in the latter situation the muscular tissue is sometimes invaded by the fungus, but only to a very limited extent. Spots that have been kept under observation for six months show a penetration into the muscle of 1 mm. only, whereas the diameter of the spots increased sixfold during this period. On the other hand, when a spot occurs on connective tissue lying on a layer of fat, the latter acts as a perfect check to the progress of the fungus ; a few stray hyphae may penetrate for a very short distance between the particles of fat and then perish (Fig. 3). The black patches invariably remain perfectly dry, that is, there is never any indication of liquefaction or of putrescence set up by the fungus.

The fungus will not grow on bones nor on cartilage.

Pure Cultures. Effect of Temperature on Growth.

When spores (conidia) were sown in a hanging-drop in water containing one per cent. of cane sugar in solution, germination took place after fourteen hours at a temperature ranging between 55° F. and 62° F. On the other hand, when the temperature did not exceed 50° F., many of the spores germinated after six hours' immersion in the liquid, and the growth was much more vigorous and rapid than at the higher temperature. In a third experiment the hanging-drop preparation was placed on crushed ice. In this instance germination took place at the end of twenty hours and proceeded slowly, although in course of time the germ-tubes were quite as strong as those produced at a higher temperature.

A second series of cultures in Petri dishes containing a gelatine and meat extract nutrient medium gave similar results to the above, germination being slowest when the temperature was maintained at 32° F. One Petri dish that had been standing on crushed ice for four days showed several minute, dark-coloured specks of the fungus about 1 mm. in diameter. This dish was then exposed to a temperature varying between 35° and 40° F. After three days at this temperature the largest spot was 8 mm. in diameter, the others being only slightly smaller; at the end of a week at the same temperature the largest spot measured 1.5 cm. in diameter. Two more Petri dish cultures were kept throughout at a temperature ranging between 35° and 40° F.; at the expiration of ten days the largest black patch of fungus had a diameter of 8 mm. Further experiments demonstrated that the growth of the fungus was most vigorous at temperatures near the freezing point and was also favoured by slight fluctuations in temperature. At higher temperatures and also at any one fixed temperature growth was slow. The optimum of growth was attained when subjected to oscillations of temperature ranging between 35° and 40° F. The fungus grows very slowly, if at all, at a temperature ranging between 60° and 70° F. Portions of spotted beef buried with the exception of the upper surface in powdered charcoal, have been kept for a period of six months during the summer season in the Jodrell Laboratory, Kew Gardens, and at the expiration of this period of time the spots had increased from two to three millimetres in diameter only, yet spores and mycelium from this material grew vigorously when placed in a suitable nutrient material and exposed to a low temperature.

This predilection for a low temperature, or for sudden changes of temperature, is by no means peculiar to the fungus causing "black spot" on chilled beef, in fact it is a trait common to many of the fungi causing epidemics in the Vegetable Kingdom. The farmer and horticulturalist are perfectly aware that what is termed "jumpy" weather in spring, that is, alternating spells of mild and cold weather, greatly favours diseases caused by fungi, whereas fairly uniform weather, whether mild or cold, is inimical to fungus parasites. It has also been demonstrated that in cases where the spores of fungi fail to germinate readily, exposure to freezing point, by placing them on ice for twenty-four hours, brings about the desired result.

A piece of beef showing "black spot" was placed for a week on a mixture of salt and ice, which may be taken as representing 0° F. Conidia from this example germinated readily when placed in a nutrient solution at a temperature averaging 45° F., whereas unfrozen conidia from the same material required a longer period of time and the percentage of germinating conidia is much lower.

When a "black spot" is removed from a piece of meat together with the underlying tissue it does not recur.

New spots do not appear on meat which is affected by "black spot" if the meat is kept at the ordinary air temperature (55° to 70° F.). If, however, the meat is kept at freezing point new spots appear after the lapse of about four days. These new spots are probably due to the germination of conidia from existing spots; if the surface of meat on which black spots are present is rubbed so as to distribute conidia the new spots are very much more numerous. If the surface of the meat is washed with a solution of sulphurous acid before rubbing no new spots appear on keeping the meat at freezing point. At this temperature the spots may reach 2 mm. in diameter in a week and afterwards grow still more quickly. Below 0° C. the rate of growth is only appreciable after the lapse of a month; if the temperature be then raised to 42° F. the rate of growth is greatly accelerated. If the meat is kept at ordinary temperature (50° to 70° F.) after the new spots have just appeared they increase to 1 mm. in diameter at the end of a week and afterwards grow very slowly.

Nature of the Fungus causing "Black Spot."

A cursory examination showed that this fungus belongs to the cosmopolitan form-genus *Cladosporium*, the various forms of which are considered as phases in the life-cycle of higher, ascigerous fungi. In

some instances such connection has been proved. All previously known species are either saprophytic or parasitic on plants. No trace of germination was observed when spores of the fungus were sown in extracts of vegetable humus, wood, or living parts of various plants, neither could living leaves be infected either on an unbroken or wounded surface. After repeated consecutive cultures from the same stock of material, in gelatine and beef extract, and at gradually increasing temperatures up to 65° F., the fungus gradually assumed the structure characteristic of the genus. The formation of dense masses of conidia on the surface of the substratum was gradually abandoned and replaced by the production of elongated conidiophores springing from a compacted mass of mycelium buried in the substratum. The aerial conidia were 1-septate, and of variable size. On germination these conidia gave origin to the secondary type of conidia known as *Hormodendron*. These characters proved the fungus causing “black spot” to be none other than the cosmopolitan and very variable form-species called *Cladosporium herbarum*, Persoon.

Source of Infection by “Black Spot” Fungus.

Judging from the frequent occurrence of the “black spot” fungus on chilled beef, and from the stage of development at which it has arrived when the meat is first unpacked in this country, it is obvious that infection takes place before the meat is shipped. The continuation of this state of things can only be prevented by the exercise of methodical measures, such as the destruction of all waste material, animal and vegetable, favouring the development of the fungus from the neighbourhood of the packing sheds, etc. The latter should also be periodically sterilised, externally and internally, by the use of a suitable antiseptic. There is no necessity if proper precautions are taken to treat the surface of the meat itself with formaldehyde or other antiseptics which are objectionable from the point of view of the meat consumer.

The material used for wrapping the carcasses in should also be sterilised before being used.

Those portions of the carcasses most liable to be attacked by fungi would benefit by being dressed with a strong solution of brine before the wrapper is fixed on.

Fungi occasionally forming Spots on Chilled Beef.

The fungi enumerated below have occurred occasionally along with the "black spot" fungus, but are comparatively unimportant. In the majority of instances it is highly probable that their presence is due to infection from spores present where the meat is unpacked, after its arrival in this country.

Oospora carneola, Sacc. This fungus has occurred on two occasions on different consignments. It forms small, dingy pink or pale flesh-coloured patches up to 1 cm. diameter, consisting of long necklace-like chains of very minute, globose cells.

Verticillium lateritium, Berk. Forms rather extensive velvety patches of a deep orange-red colour. Only seen once.

Penicillium glaucum, Link. The blue-green mould so common on stale bread, cheese, jam, etc., in this country, has occurred on several occasions both on the meat and on the wrappers. Infection may possibly have occurred after the arrival of the meat in this country.

Penicillium candidum, Link. Forming small snow-white mealy cushions up to 1 cm. in diameter. Closely allied to *P. glaucum*, differing more especially in the chains of minute globose spores remaining permanently white, instead of changing at maturity to a pale blue-green colour as in the last-named fungus.

Mucor mucedo, L. Found once.

Phycomyces nitens, Kunze. Occurred once on fat. Probably infection occurred in this country, although this fungus is widely distributed. Quite superficial in its growth, and leaving no stain on the meat when rubbed away.

Mucor racemosus, Fres. Present once in very small quantity.

The remaining species were found in very small numbers. A few undescribed forms of academic interest only, will be dealt with elsewhere (*Kew Garden Journal*).

SUMMARY.

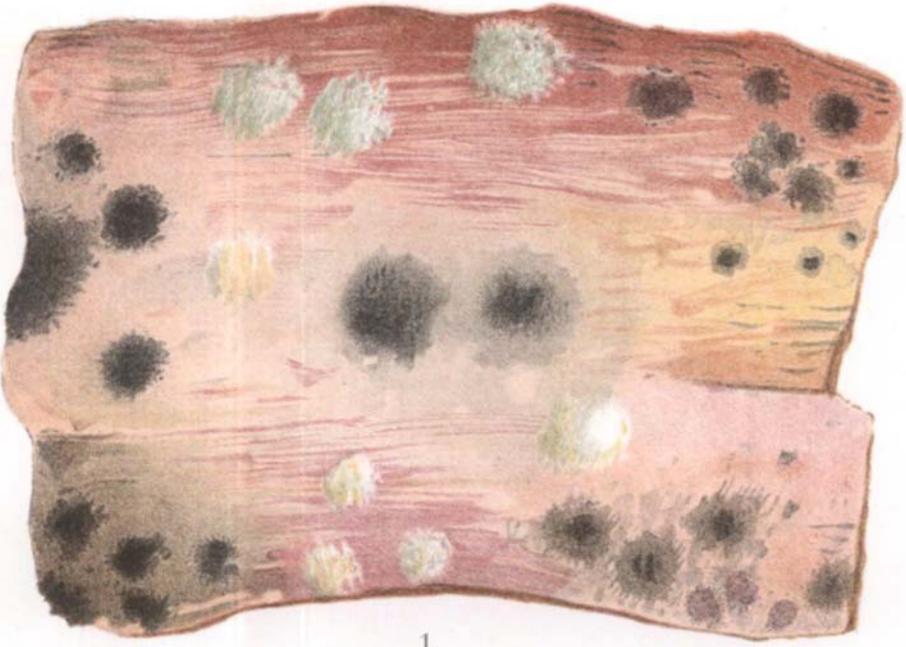
The variously coloured patches appearing on the surface of chilled beef are caused by fungi. Amongst these, *Cladosporium herbarum*, producing black spots, is the only fungus that has become specially adapted for flourishing on such an unusual substratum, and may in consequence be expected to continue and even increase in quantity on its new matrix, unless stringent measures are adopted. This fungus is

not pathogenic, and the only effect caused by its presence is the unsightly appearance of the meat and consequent deterioration in value. If extensive, the meat may be rendered unsaleable.

The beef is infected with the "black spot" fungus before it is shipped, and to arrest the continuance of this infection special measures should be taken, with the object of exterminating the fungus, which in its specialised form can only occur in the vicinity of the establishment where meat is prepared for shipment.

DESCRIPTION OF PLATES VI AND VII.

- Fig. 1. Portion of chilled beef showing crowded patches of "black spot" and other fungi. Natural size.
- Fig. 2. Portion of chilled beef showing scattered patches of fungus, *Cladosporium herbarum* Pers. Natural size.
- Fig. 3. Section through subcutaneous areolar tissue resting on fat. The mycelium of the *Cladosporium* is abundant in the subcutaneous areolar tissue but penetrates the fat very sparingly. $\times 400$.
- Fig. 4. Section through the subcutaneous tissue, showing the mycelium of the *Cladosporium* producing secondary spores at the surface of the substratum. A few rudimentary conidiophores are growing out into the air. $\times 400$.
- Fig. 5. Mycelium of *Cladosporium* with chlamydo spores. $\times 400$.
- Fig. 6. *Cladosporium herbarum* Pers., the typical form with aerial conidiophores bearing conidia. On the right, conidia germinating and producing the *Hormodendron* stage. On the left, chlamydo spores germinating. $\times 400$.

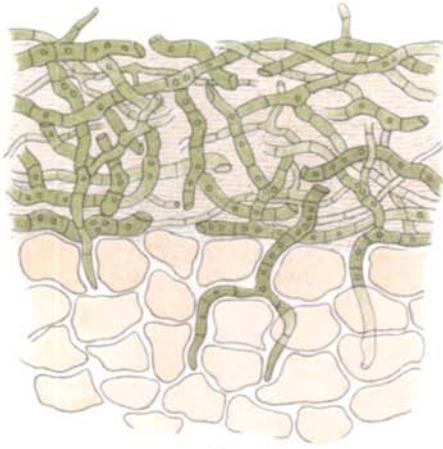


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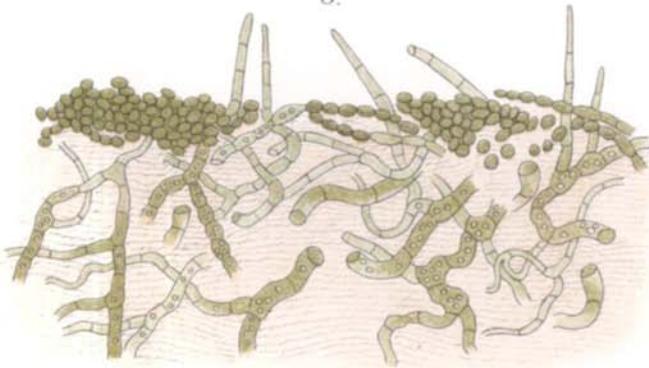


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E. Wilson, Cambridge.



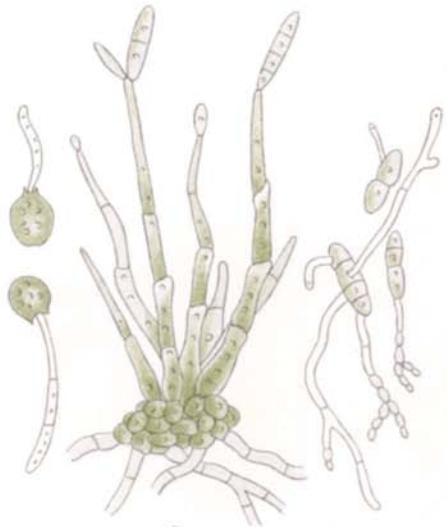
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