I had the satisfaction of exhibiting several times to Mr. Reade, the upper and lower beads of the test-scales he brought with him, and we were both greatly pleased with the beautiful, I might say exquisite, colouring of the rows of beads or rouleaus, according as they were in the upper or lower plane. Not less remarkable were the prominent and *enlarged* appearance of every fourth or fifth bead of a vivid azure blue: a phenomenon capable, no doubt, of good physical demonstration.

The *azure blue scales* described in the paper are similar in structure to the *Macrotoma Major*; but very finely marked, more delicate and transparent.

Altogether this new test promises a new field of research.

IV.—*Cultivation, &c., of Microscopic Fungi.*

By R. L. Maddox, M.D.

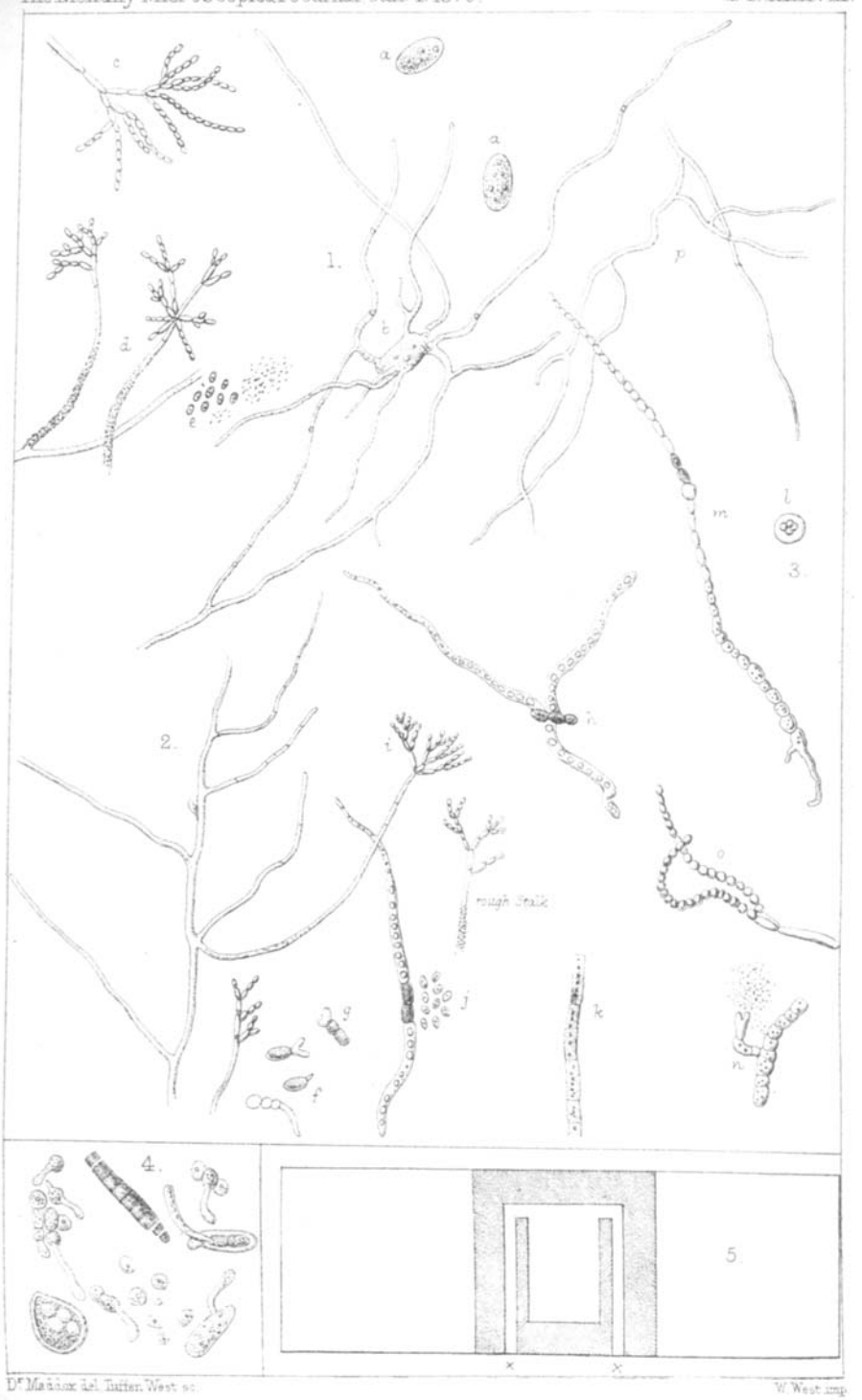
Plate XXXVIII.

The cultivation of Microscopic Fungi offering such a wide and tempting field for research, and the few experiments conducted in reference to "Mucor Mucedo," as stated in a previous article, p. 140, vol. ii. of this Journal, being incomplete, others were set forward without delay; but unfortunately, from unavoidable absence from home shortly after, they must for the present be entirely passed

---

EXPLANATION OF PLATE.

Fig. 1.—*a. a.* Spores or conidia of *Oidium Tuckerii.*
b. The same, germinating.
c. Pedicels with fruit.
d. Older stalks, rough on the outside.
e. Small nucleated spores and bacteria.
„ 2.—*f.* Spores or conidia from brand on orange leaf.
g, h. The same, germinating.
i. Stalk with spores—one—rough on the outside.
j. Small spores nucleated.
k. Part of an old mycelial thread, with minute bodies enclosed between the septa.
„ 3.—*l.* Spore or conidia from brand on leaf of a climbing plant.
m. The same germinating and producing irregular cells.
n. Terminal cell supposed to have discharged the minute bodies.
o. Moniliform rows of *Penicillium glaucum.*
p. Mycelium from the brand on the leaf.
„ 4.—Figures of the bodies now found (Dec. 13) in *the two large drops* of juice alluded to in pp. 144, 145, vol. ii., of this Journal, representing the true spores, the small schizonematous bodies and minute granules or molecules, and which show them to appear at least as fungoidal elements.
„ 5. Cultivating slide.



Cultivation of Microscopic Fungi

over, being too imperfect for publication, and others only slightly sketched.

As the subject is likely to occupy considerable attention at home and abroad, and different observers employ different plans, I venture to shortly notice the method adopted by myself, which proved useful and inexpensive, and allude more particularly to that employed by Drs. Billings and Curtis, as stated in their portion of the late "Report on the Cattle Plague in America."

The great difficulty, if not almost absolute impossibility, of conducting any series of experiments, which, however free to legitimate deduction, shall not be open to useless controversy, determined me to use only such means as might be called simply precautionary, of easy arrangement and manipulation, one object being to determine some useful form of cultivating-slide with the ordinary 3-inch microscopic object-slide. Several plans were tried, but the one now described selected. It was made as follows, to be used with thin $\frac{6}{8}$ths of an inch square covering-glass, such as is usually employed for a $\frac{1}{12}$th-objective.

A piece of tin-foil, of the stoutness of ordinary note-paper, was cut into squares of one inch diameter, a portion was then removed from a square by cutting it from one side almost to the opposite, then at right angles and again at right angles, thus leaving a strip of the shape of the letter ⊔ with a flat base, and limbs of equal length and width; from the piece removed a narrow slip was cut from three sides, and then a portion removed from it, as in the first piece, thus forming a smaller letter ⊔; the largest was cemented to the surface of a well-cleaned slide with easily-melting marine glue, or Bell's cement thickened with gum-shellac; the smaller one was then similarly fixed within the larger one, the limbs being turned to opposite sides of the slide, as seen in the figure, thus affording a central space for the material under examination and a narrow channel each side for the admission of air.

The fungi or spores selected were placed on the cover, under the dissecting or erecting microscope, with a *droplet* of the medium to be employed or cultivating solution. This was examined under the microscope with a power of 150 or 200 diameters, and if satisfactory, placed at once over the central space of the slide (which, after cleaning with liquor potassæ water and alcohol, if cemented with marine glue, or diluted alcohol if with Bell's cement, was kept turned upside down, one edge resting on a piece of glass until wanted), and the edges of the thin cover resting on the slip of tin-foil, were then closed up with wax softened by oil or with Bell's thin cement, except at the spots corresponding to the two narrow passages marked * * in the figure. If thought necessary, a duplicate slide was similarly prepared, one being left in diffused light, the other in darkness in the moist chamber.

The medium should *not touch* the inner edges of the tin-foil at any part.

To procure a satisfactory moist chamber occupying but little space, the slides were set in small porous battery cells, previously thoroughly cleansed and moistened with freshly-boiled water, and set in a basin of the same to the depth of half an inch, the basin, with levelled edges, being covered by a plate of clean glass. Those slides intended for diffused light were placed in cleaned damp white porous cells set in water as the others, and covered with glass, the face or cover side of the slide leaning towards the inner surface of the battery-cell; a small cell would thus hold four slides occupying little space. The external temperature, if not sufficiently high, must be raised artificially by a water-bath, or the basin set in a warm place.

The slides when removed are bedewed with moisture; but if rested on end, in a clean tumbler (warm if necessary), and covered with a bell-glass, this soon disappears, and permits examination with any power up to a $\frac{1}{12}$th.

Although the slide and cover were generally fogged with moisture, I sometimes found the droplet had considerably diminished, and the slide presented surfaces barely moist, especially if cover and slide had not been most carefully cleaned. To meet this difficulty, which was somewhat serious, especially if the slide had to be watched many days, it occurred to me that if an artificial cultivating fluid was made, which would be hygrometric, besides containing the elements of nourishment considered useful for the colour and growth of the fungi, much of this difficulty might be overcome; hence, after several trials, the following was selected and successfully used:—

Dextrine, 2 grains; phosphate soda and ammonia, 2 grains; saturated solution of acetate potash, 12 drops; grape sugar, 16 grains; freshly-distilled water, 1 ounce; boiled in a clean glass vessel (thin beaker or large test-tube) for 15 minutes, covered whilst boiling and cooling; when settled poured into perfectly clean two-drachm stoppered bottles, and set aside for use. Sometimes with the cultivating fluid other media were added on the slide.

To preserve some of the perfect forms of fungi found on plants, or produced by cultivation, &c., a saturated solution of acetate of potash was employed with success. There is, however, a little difficulty often from the repellent nature of the heads when beset with spores, and retaining air in their interstices, in using this medium for mounting; but flooding the specimen momentarily with alcohol diluted to the point when it readily touches or wets all the surfaces, and draining it from the slide before applying the mounting solution, readily overcomes this little trouble, and with scarcely any appreciable change in the appearance of the spores or mycelium; at least such is my experience. I prefer it to glycerine or any other solution

used. The edges of the cover, if wetted by the acetate solution, attract moisture readily; so it is best, if the specimen be worth closing up, to dry the edges by a sable pencil and twist of tissue-paper before applying Bell's cement, at first thin, then thickened, as aforementioned.

On the 1st September, a few of the spores (conidia or sporanges) were removed from the surface of a grape, covered with *Oidium Tuckerii*, and sown as described in the cultivating solution, placed in the porous cell in the moist chamber, and left in diffused light. On the 4th there was an abundant mycelium from several of the spores or conidia (Fig. 1 *b*), afterwards becoming of a light-brown colour, divided by septa, enclosing generally two oil globules or minute spores. Later, on the 15th, from the mycelium several stalks had sprouted, and fruited into the air-space beyond the edge of the liquid; the fruit resembling Penicillium, but the spores in rows on the pedicels were slightly oval. Some of the older stalks had a character which I had not noticed before, and which I have endeavoured to show in Fig. 1 *d*. They appeared rough outside, or covered with most minute bodies. I am of opinion that this was not an accidental character, but from absence was unable to trace this point further, or whether such stalks might not break up into other bodies.

Lying about amongst the threads were numerous minute bodies (?)bacteria, also small oval bodies with a central spot or nucleus, mostly collected into groups (Fig. 1 *e*). It is possible these may have been drawn in from some slight shrinking of the fluid, and have originated, as supposed, from the heads that fruited in the air-space; but whence they were derived I am uncertain.

On August 30th, a small speck from a brand on an orange-leaf (from the same greenhouse), which for trial had been set in fresh gooseberry juice, was removed, as it had not altered, and set in the cultivating solution, &c., in the light. The spores or conidia commenced sprouting very shortly after, and soon became filled with oil globules or spores, as in Fig. 2 *g*, *h*. On the 5th many of the mycelium threads had formed heads outside the fluid, the spores being very slightly oval (Fig. 2 *i*).

In the fluid lying by the side of some of the original conidia or spores which had sprouted were minute oval bodies with a central nucleus in each (Fig. 2 *j*). On the 15th the stems of the older fruited threads appeared roughened on the exterior, with minute bodies, as in the former case. The young threads were growing beautifully, and filled with closely-packed oil globules or spores, no septa being distinguishable, while in the older mycelial threads, which were of a brownish colour, septa existed; each division inclosing about six small bodies (Fig. 2 *k*).

Another slide was set from a black brand (found on a leaf of a climbing plant, * * *, in the same greenhouse) with the culti-

vating solution on the 1st September; on the 5th some of the conidia or sporanges showed in their interior four distinct spores (Fig. 3 *l*); some of the loose spores gradually sprouted, forming threads consisting of irregularly-shaped cells, many of them containing from one to four, either oil globules or spores (Fig. 3 *m*); these, like the former, were not tested with ether, &c., as it was desired to preserve the specimen intact. On the 15th the threads were full of these, and along their edges thousands of bacteria-like bodies were present. I am doubtful if these were not ejected from the sporiferous (?) ends of some of the offshoots, as in Fig. 3 *n*. Though I had not witnessed their ejection, their position and the appearance of the terminal cells in some parts led to this supposition. The heads with spores in the air-space resembled *Penicillium glaucum* with its moniliform chain of round spores, and were larger than in the other specimens (Fig. 3 *o*). The mycelium from the same brand is seen in Fig. 3 *p*. At this interesting point of inquiry they were obliged to be neglected, and are only alluded to here in this imperfect sketch, rather to show the utility of the cultivating slide and solution than otherwise; for in the two last experiments it would be difficult to prove more than that the fruit sprang from some particular spore of those found in the brand, which possibly might be of a mixed character.

In the very valuable and suggestive report of experiments by Drs. Billings and Curtis, of the U.S. army, appended to the comprehensive and extended researches furnished by Prof. John Gamgee, M.D., on the cattle plague in the United States, under the various titles of "lung disease, or pleuro-pneumonia," "the ill effects of smutty corn on cattle," and "the Texan, periodic or splenic fever," undertaken by the authority of the commissioners of agriculture, we find the microscopic examination and the cultivating experiments handled in a very careful, instructive, and trustworthy manner. The omission of any experiments carried out in *darkness* in reference to the cultivation of fungi from the healthy or pathological tissues or fluids, more especially those relating to the most serious and difficult question of the cause of Texan or splenic fever, which destroys life so largely, is to be regretted, as it leaves this point open for future investigation.

Experiments performed outside the body, if darkness be omitted as one of the conditions, however carefully temperature and moisture may have been regarded, may not sufficiently closely imitate the natural relations, and determine a difference as regards the results obtained. This is not to say that such differences do exist, nor is it to be expected that all the circumstances can be rendered similar, hence there may remain always some degree of questioning, but this condition adopted would tend to narrow the circle. My object is not to enter into a review of this most interesting and valuable

Report,* but for the benefit of those who may be engaged in or undertake such examinations, it may be useful to give a brief statement of some of the methods employed, and the results arrived at by those excellent observers.

The questions they have endeavoured to answer are:—

1st. "Are any forms of cryptogamic growth present during life in the blood or secretions of diseased animals?"

2nd. "If so, of what character are they, and what is their probable source?"

All relation between the cryptogram and the disease, as cause or effect, being neglected.

The supposition by some, which they give succinctly, is that "disease is produced by the presence in the economy of minute particles of protoplasm (micrococcus of Hallier), resulting from development and breaking-up of the spores or mycelium of a fungus; from which granules, they assert, can be developed perfect forms of fungi, of recognizable genera and species, by proper 'cultivation' outside of the body of the animal fluids containing them." In the fresh venous blood from a pleuro-pneumonic cow under 1200′ diameters, they found no unusual appearances to healthy blood as regards corpuscles, spores, or mycelium, but, "single or in masses," minute granules or molecules were seen in the field as "glistening points," if not at first, at least after exposure to the "air for a few hours." These particles have been claimed as the course of disease, pronounced to be vegetable in character, and "as being developed from, and capable of reproducing certain common fungi popularly known as rusts, smuts, or molds."

The fluids were obtained in a state of purity, by using short glass tubes about $\frac{3}{16}$ths of an inch in diameter, made by sealing one end at the flame of a Bunsen burner, holding the tube in it nearly upright with pincers till it is red hot, rapidly drawing the tube out to a narrow neck and closing it in the flame; the hermetically closed tube with a partial vacuum is called a "vacuum tube." The insertion of the point into a vein, compressed above and below, when broken off allows the blood to enter, and the tube on withdrawal is immediately sealed by the flame of a spirit lamp. The fluid can now be kept for experiment without the entrance of foreign spores; but to place any portions of this in conditions necessary for cultivating without risk from their entry, offers a difficulty, upon which those observers reasoned as follows, and which so entirely corresponds with my own views as expressed in another paper, that I venture to quote their words.

"By no amount of precaution or complexity of apparatus is it possible to secure such absolute isolation of a fragment of tissue or

* Reports of the diseases of cattle in the United States, made to the Commissioners of Agriculture, Washington, Government Printing Office, 1869.

a quantity of blood from possible contact with foreign spores, that the results obtained from its cultivation can be considered as positively conclusive. By no means known to us can a piece of lung be transferred from the body of an animal to the interior of a glass flask, without contact with the atmosphere and with instruments, nor even with the more manageable blood can we be absolutely certain when we see its surface covered with mold, that the possibly single spore from which that forest sprang must infallibly have been in the vein of the animal whence the blood was drawn. It was felt, therefore, that to adopt at the outset extraordinary precautions against the introduction of foreign spores, would be more apt to lead to error than even taking none at all. The method of comparison was therefore resorted to." Thus healthy and diseased tissues and fluids were similarly treated, using ordinary precautions.

The "isolation" apparatus they adopted is a thin, flat-bottomed flask, with a cork dipped in paraffin at the neck, pierced for a tube bent at right angles, closed at the outer end with a plug of fine cotton wool.

The culture apparatus used was a large flat glass cell, containing a porcelain stand, rather higher, which supports a glass shelf for holding the slides and watch-glasses for daily examination, this covered by a glass bell jar closed at the neck by a cork dipped in paraffin, through which is passed a right angle bent tube, the outer end plugged with cotton, and the interspace between the outer cell and glass jar filled with a strong solution of permanganate of potash. These somewhat, as stated, resembling Hallier's, but no means were added for drawing fresh air into the vessel.

The growing slide recommended is the ordinary 3×1 inch slide, having "a piece of thin, fine, white blotting-paper of the same size, with an opening in the centre three-fourths of an inch in diameter, or a little less than that of the thin glass cover used. The edges of the paper may be cemented to the glass with a little Canada balsam, although this is not necessary. To use it, put it in strong alcohol for ten minutes, then in distilled water for the same length of time; free the central opening from water; place in it a drop of the fluid to be cultivated, and cover it with a thin glass cover." It is to be kept flat and set in a culture apparatus: when "water alone is used as the isolating fluid," a piece of sewing-thread rests on one end of the slide, the other end dipping into the water. If not to be set in a moist chamber, the paper is covered with a corresponding "piece of thin sheet rubber or oiled silk," with a similar central aperture. If the fruit is to be developed, "a groove should be cut in the paper to the edge of the slide" to admit air. A very ingenious form of development apparatus used was a glass beaker containing a little water, closed at the top by a thin sheet of rubber having suspended from its centre, by a thread, "a strip of thin

blotting-paper which had been previously soaked in alcohol and distilled water, and on which the material to be cultivated had been placed." Perhaps a similar employment of a few long fibres of asbestos might be useful, as they could be heated red hot so as to destroy any germs, and if the object to be cultivated was placed half-an-inch from the end, this might be allowed to dip into a small cell having the necessary cultivating solution, and the cell itself surrounded by water for a moist chamber.

The substrata employed for the nourishment of any fungi present, were of various kinds, natural liquids, animal and vegetable, also solutions of sugar, cane, and grape, and solutions of tartrate of ammonia, ashes of yeast and water, &c., &c., some boiled, and if filtered, reboiled.

The experiments are numerous and most interesting, and the conclusion derived from them is "that, in the contagious pleuropneumonia of cattle, there is no peculiar fungus-germ present in the blood or secretions, and that the theory of its cryptogamic origin is untenable."

With the blood from the splenic disease, "which was placed in various substrata, and compared with healthy blood, the results were in all cases the same, *i. e.* production of penicillium, coremium, and mucor."

They found in the blood, bile, and urine of animals slaughtered in Texas, "apparently healthy while alive, yet after death" presenting characters of splenic fever, "minute bodies corresponding to the micrococcus of Hallier, which exhibit the same behaviour with reagents as the spores of fungi."

These micrococci are undistinguishable from "similar bodies" found in "any blood in an incipient stage of putrefaction." More, over, cultivation, in various ways, of the blood containing them failed to invest them with a special and important character. The growths were "composed of the commonest mold, and instead of being unique as to species or even genus, comprised various forms and sizes of cryptococcus, torula, penicillium, coremium, mucor, and the so-called schyzosporangia of Hallier" "either simultaneously or successively developed." Healthy blood they found to yield the same results, but more slowly.

Ustilago, coniothecium, or tilletia, were not obtained, and as said, "probably due to the circumstance that *no specimens of these fungi were ever brought into the room where our experiments were conducted*" (the italics are ours). These cautious observers thus deduced the hypothesis from their experiments, the cultivation yielding only the commonest molds, "that the disease rather destroys the vitality of the blood to such a degree as to render it capable of supporting and nourishing a low form of these ubiquitous fungi, which perish when introduced into a healthy subject," and

suppose these granules, if fungous in their nature, should be considered rather "as an effect of the malady, whether constant and inherent, or altogether fortuitous," "than imagine a deadly disease occurring only under certain rigidly prescribed conditions, as caused by the presence, in the economy of the germs, of fungi notoriously harmless and of universal occurrence." They admit the possibility of these fungi in the fluids of the diseased animals becoming the "carriers of contagium."

The Rev. M. J. Berkeley, to whom we are so largely indebted for our knowledge of microscopic and other fungi, in his paper, page 14, vol. ii., of this Journal, thus expresses himself in reference to these small bodies: "But, whatever may be the origin of these minute bodies in question, whether from pre-existent spores or the fortuitous concourse of chemical and other energetic forces, it is a matter of immense importance to ascertain whether they have any real connection with disease, and it is at once obvious that the question as to their origin becomes eminently essential." Everything, therefore, which may help forward the difficulty, though it may not overcome it, has its appreciable value.

It is, perhaps, at this point of interest to notice the curious results marked by Professor Gamgee of cattle driven over the trail of Texan cattle, which themselves may have shown no signs of the disease while alive, conferring the disease upon the new comers in a most fatal manner, yet the survivors of those "animals contaminated by feeding on Texan trails have not in a single instance propagated the disease to other animals," &c., nor do the originally infected cattle occasion the disease by actual contact. Professor Gamgee remarks, it is "not the breath, nor the saliva, nor the cutaneous emanations which are charged with the poisonous principle, but the fœces and the urine." The conditions, he states, are modified by weather, season of year, and time. This alone, considering the sad waste of life and actual loss of property to those engaged in the produce of stock, or as "packers" for a vast consumption in their own country or in others, where preserved meat may, under emergency or price, influence the markets, is a field of research that cannot be too widely investigated. From the observations of others, speaking of the ill effects of diseased corn in cattle fed with it, he remarks that pigs "acquire a taste for it, and after eating it a few days their bristles drop out, there is an awkwardness in the movements of the hind legs, and atrophy affects them. Eating the pigs produces no ill effects on man." "Hens lay eggs without shells;" monkeys and parrots fall down, "unable to rise again." "The indigenous dogs and deer that enter the corn-fields at night suffer in the same way;" yet, in an experiment, two cows were fed with food, somewhat dry in one case and wetted in the other, and mixed with smut fungi; the only effect observed was,

"the cow fed on the dry food lost flesh," on the "wetted food gained in condition." They consumed forty pounds of smut in three weeks, the appetite being voracious. Thus we find how much we have to learn amongst the diseases of cattle, and how such important investigations amongst inferior beings may tend to unshroud the contagious, endemic, and epidemic maladies that encircle ourselves. We have paid too little attention to the excreta in large and small communities, and here we see opened before us, by vast and devastating results, an inquiry of how far the statement that the Texan or splenic fever, "an enzootic disorder, probably due to the food on which Southern cattle subsist, whereby the systems of these animals become charged with deleterious principles, that are afterwards propagated and dispersed by the excreta of apparently healthy, as well as obviously sick, stock," may prove correct; and whether, under a constant inquiry relative to zymotic diseases generally, the importance of experimental development of fungi might not be more seriously urged in this and other countries.

Dr. Gamgee says that the spring grasses, after the frosts of winter have killed the old ones, are healthy, and continue so unless Texan or Florida cattle are again driven over them. Mr. H. W. Ravenel, the accomplished botanist of South Carolina, who accompanied Dr. Gamgee, found no parasitic fungi on the young grasses or hay at the time of their visit, that could in any way account for the disease, nor did he find the coniothecium Stilesianum which Hallier suggested should be "looked for in the food of the wild bullocks."

Besides the foregoing brief notice of the methods employed by Drs. Billings and Curtis, it is necessary to allude shortly to another which may yet have very important bearings in the solution of some points difficult to study; they carried out a series of experiments in reference to the passage of "bacteria, vibrios, and molecules, either single or in chains (*Monas, Microzymas, Micrococcus, Leptothrix, Zoogléa, and Schizomycetes of various authors*)," through thoroughly moistened filtering paper, while, as originally shown by Mitzscherlich, "yeast cells will not pass," and that none of the aforementioned bodies pass through vegetable parchment, though this is open to the passage of fluids; the apparatus used being simply a test-tube open at both ends, one end is now closed by doubled strong filtering paper, tied by waxed thread, which end is rested on a glass rod inside a four or six ounce glass beaker, rimmed, and after certain precautions the fluids for operation were placed, the one in the beaker, the other, the putrefying or fermenting fluid, in the tube, and the beaker finally closed by lightly stretching and fastening sheet rubber over the top: the solutions and slides, prepared with the same fluids, were very numerously varied; putre-

factive fluids determining the formation of yeast-cells in the external fluids which previously contained none; in other cases yeast-cells being found in the tube and molecules or micrococcus in the beaker. From these many and varied experiments, they consider it probable "that some of the bacteria and micrococcus germs are really fungoid in character, and capable of being developed into higher forms."

Although they found no fungus germs in the blood of many healthy and diseased animals, in others, germs existed in the blood during life, as they developed in the blood in the "vacuum tubes" filled from them; but they question whether those germs would be developed without some "dead organic matter as a pabulum." The common mildews are stated to stand, in point of frequence, thus: penicillium, then mucor, next aspergillus, these varying as to growth, colour, size, &c., in many ways.

The conditions under which bacteria and the minute germs of fungi germinate in the living economy of either vegetable or animal are very imperfectly known, and probably are at the first very trivially altered from the normal state; but once permit these minute bodies a footing, as it were, in the economy, and if retained at any one point or organ rather than another, from the rapidity of development, serious effects might be expected—(according to some botanists, cells in some fungi can be produced at the rate of 96,000,000 per minute, see Peunetier, 'L'Origine de la Vie,' p. 27) —lead to rapid decay in the cells and tissues, the formed material supplying the "dead organic matter" for their pabulum; then the secondary deposits may become deficient, and the catalytic changes induced by their presence so destroy the relation or balance of those efforts, at harmonic action, which are present in slight deviations from health, that increased sickness may follow.

That the protoplasm itself is converted into bacteria or fungoid germs is to me doubtful. In the petals of flowers, especially *Escholtzia*, some of the cells may often be seen filled with these moving bodies, supplanting the place of the normal plasma, gradually extending their domain into neighbouring cells and hastening decay; yet we are not in a position to say such germs were not introduced from without either by the spongioles of the rootlets or by the stomata. The duration of their life is also undetermined, and, if at all in proportion to the rapidity of their increase, must be short; but if originally reserved for ulterior uses, this may be reversed, and their power of resistance to increased temperature, &c., reach far beyond the point at which higher organisms perish. The cumulative evidence at present does not appear to sufficiently preponderate either way, to settle this controversial and difficult question.