

degree of specialisation of its outer portion, but no sign of any transformation of the ameloblasts themselves into any intermediate form of tissue has been observed.

The sudden and entire transformation of the ameloblasts in the Gadidæ may perhaps be correlated with the very early and rapid formation of the enamel, which is formed while there is yet but little dentine calcified.

In mammals enamel formation is a very much slower and more gradual process, and the dentine is always much further advanced towards completion than is the enamel.

A comparison of the various processes now known as occurring in fish, in implacental mammals, and in placental mammals may, in the author's opinion, be taken as finally disproving the idea, which is still entertained by some, that enamel is to be regarded as a sort of secretion shed out from the ends of the ameloblasts; for, imperfect though our knowledge remains in some respects, yet some form of conversion, direct or indirect, of a pre-existent organic matrix is common to all, though as in placental mammals it may be exceedingly small in amount, and the erroneous idea alluded to has proceeded from the study of the process exclusively in placental mammals, in whom its true nature is most difficult to decipher.

"Further Observations on 'Nitragin' and on the Nature and Functions of the Nodules of Leguminous Plants." By MARIA DAWSON, B.Sc. (Lond. and Wales), 1851 Exhibition Science Research Scholar. Communicated by Professor H. MARSHALL WARD, F.R.S. Received December 5, 1899,—Read January 18, 1900.

(Abstract.)

In December, 1898, a paper by the author on "Nitragin, and the Nodules of Leguminous Plants,"* was read to the Royal Society. Since that time the work on this subject has been considerably extended, and a brief summary of the additional results is given below.

Investigations have been made in the following directions :—

A. *Microscopic Observations.*

A comparative study of various points of interest in the anatomy of nodules borne by several genera of different tribes of the order, with special reference to the mode of growth of the nodule organisms within the tissues of the host.

* 'Phil. Trans.,' B, vol. 192, pp. 1—28.

B. *Experimental Work.*

α. Pure cultures of the organisms from *Pisum*, *Desmodium*, and "Nitragin," upon various media, liquid and solid, organic and inorganic, employing the ordinary methods of bacteriology.

β. Direct observations under the microscope of the various stages of growth of colonies, and the formation of bacteroids from straight rods, as seen in hanging drops.

γ. Experiments upon the effect of temperatures above the normal upon the direct infection of pea roots.

δ. Cultures of various genera, representing different tribes of the order, to test the power of organisms proper to one genus to induce tubercle formation upon individuals of other genera or tribes.

ε. Crop cultures in the laboratory greenhouse of peas in sterilised media, with and without inoculation with "nitragin," also with and without a supply of nitrogenous food.

ζ. Crop cultures of peas in ordinary garden soils and in subsoils, in the open, with and without inoculation with "nitragin," also with and without an additional supply of nitrates.

A further study of the morphology of nodules from various genera of the Leguminosæ, leads to the conclusion that no definite line of distinction can be drawn between genera in which filaments occur in the nodules and those in which they have not yet been observed. Several examples were found of fragmentary portions of filaments in the cells of very young nodules, whilst in older specimens these filaments were quite absent (*e.g.*, *Phaseolus*, *Desmodium*, *Acacia*, and others), suggesting an intermediate stage in the adaptation of the parasite to the special conditions existing in any given host.

During the course of this study, some peculiar anatomical characters have been observed in certain nodules, *e.g.*, the presence of a definite crystal layer in some genera, of apple-green nucleus-like bodies in *Desmodium* and *Robinia*, and of organisms of an unusually large size in *Desmodium*, *Coronilla*, *Psoralea*, and others.

A prolonged study was made of the organisms from *Desmodium gyrans* in particular. Pure cultures were obtained, and from these observations in hanging drops upon bacteroid formation showed that the X and Y forms arise *by distinct lateral branching of the straight rods*.

After twelve to fourteen days' culture, the individual long rods tend to break up into small rodlets, and the branched forms become dis-jointed in a similar manner. A general study of these organisms and parallel cultures of "nitragin," compared with pure cultures of organisms obtained direct from *Pisum* tubercles, shows that they all alike grow readily on gelatine or agar media containing an extract of pea stems, asparagine, and sugar, but very slowly on broth gelatine. They do not peptonise milk, but upon potato a watery streak is formed

in about five days; in a liquid medium—pea extract—a thick, zooglœa-like film forms in twelve to fourteen days. The presence or absence of spores in these films is now under investigation. The organisms are aërobic, and may pass through a short motile stage, but the presence of cilia has not been demonstrated. On a medium consisting of silica jelly and a mixture of salts, including ammonium sulphate, abundant growths of the organisms from *Pisum* and *Desmodium* have been obtained; also in hanging drops of silica jelly, colonies of the latter type have grown to 30 μ diameter in seven days at 17° C. Further experiments are now in progress in order to test whether these organisms are *per se* capable of (a) fixing free nitrogen, or (b) converting nitrogen in the form of ammonium salts into nitrites or nitrates; also to determine whether or not the presence of nitrates in the culture medium is directly injurious to the organisms. At a temperature ranging from 24—35° C. (average 30°) a considerable increase in the percentage of direct infections of pea roots was obtained, but at temperatures above 35°, the host plants themselves succumbed after fourteen days. In water cultures only very early stages of infection were observed.

Experiments to determine the action of the organisms proper to one genus upon plants of another tribe or genus suggest that there is probably only one organism capable of producing nodules on leguminous plants, but that in each particular host special physiological conditions exist, to which the organisms become so specially adapted as to make it difficult for successful reciprocal action to take place between plants not nearly allied, though exceptions do occur.

In connection with infection experiments conducted under, as far as possible, sterile conditions, it was determined that fifteen minutes' treatment of seeds with a 0.1 per cent. solution of mercuric chloride before sowing, is without injurious effect upon the seeds, but that a longer action of the solution poisons the embryo. Crop cultures in *sterilised* media give best results when nitrates without organisms are supplied to the plants. The addition of "nitragin" under these conditions is of very little benefit, and if a sufficient supply of nitrogenous food be available, a reduction in the resulting crops ensues when this "fertiliser" is employed.

In *unsterilised* media a small increase in crop may result from the use of "nitragin." The conclusion derived from the various experiments, however, is that the presence or absence of "nitragin" is but one factor in a complex problem, and that at the same time must be taken into account the complicated physical and biological conditions of the soil and atmospheric environments, as well as the symbiotic action of the host plants, in the removal of the products of metabolism from the field of action of the nodule organisms.