LVIII. THE DISTRIBUTION OF INORGANIC IRON IN PLANT AND ANIMAL TISSUES.

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Experimental Methods.

In carrying out tests for the detection of inorganic iron in the tissues, two points must be borne in mind, firstly, the previous preparation of the tissue, and, secondly, the application of the reagents so that false results are not obtained.

With plant tissues when the question is not the structural arrangement but rather whether this or that cell-constituent contains iron, it is better to work with finely teased or broken-up tissues. Glass rods, drawn to a point, were used for this purpose, and also in order to break up some of the green cells and set free the chloroplasts, a portion of the tissue in each case was still more broken up by turning upon it the blunt end of the glass rod and grinding it between this and the microscope slide on which it was being mounted.

In tissues which contained a large quantity of chlorophyll, or where it was desired to stain the stroma of the chloroplast for inorganic iron, the tissues were first boiled in absolute alcohol several times until they became colourless, the greenish extract being poured off with each fresh quantity of alcohol.

In the case of animal tissues, organisms of small size can be mounted direct, but with larger tissues sections have to be cut. The tissues for this purpose were hardened in absolute alcohol for 24 hours, and then embedded in paraffin.

The sections were mounted on slides and after removing the paraffin by xylene, were "taken down" the alcohol-water series before staining.

Preliminary observations were made upon plant tissues, with the ammonium sulphide and ferrocyanide methods.

Ammonium Sulphide Method.

This method was introduced by Vogel in 1845. The solution used was one containing ammonium hydrogen sulphide, prepared by passing sulphuretted hydrogen through dilute ammonia having a specific gravity of 0·96. The teased tissues were placed in this solution (which must be freshly prepared) for three or four hours, and then mounted in the stain, ringing the coverslip with gold size.

The inorganic iron in the tissues is stained brownish-black, while the rest of the tissues are stained a lighter brown.

The chief disadvantages of the method are: firstly, the difficulty of recognising the iron when present in small quantities, because, the rest of the tissue being stained brown, it is only by very careful comparisons in tissues of equal thickness that the presence of iron can be detected with certainty; and secondly, permanent sections can only be made with difficulty.

Ferrocyanide Method.

This method was first used by Perls [1879] and ater by Molisch [1893], who was the first to differentiate between inorganic and "masked" iron.

The tissues are placed in a freshly prepared solution of 1·5 % potassium ferrocyanide and a 5 % solution of hydrochloric acid, for three or four hours, and then mounted in the stain and the coverslip ringed with gold size.

It is advisable to use ferrocyanide solution rather than ferricyanide, as the iron is chiefly present in the ferric form, and consequently then gives "Prussian blue" at once. When treated in this way the tissues tend to vary in their depth of staining, this becoming more intense during the first few days, especially if exposed to day-light, and then gradually fading to a much lighter colour.

Another disadvantage of this method is, that if permanent sections are made in Canada balsam, they fade rapidly, this being probably due to the reduction of the stain by means of the balsam forming a compound analogous to $Fe_2Fe(CN)_6$, which is white in the absence of oxygen [Mann, 1902].

Haematoxylin Method.

This method, which was introduced by Macallum [1897], depends on the fact that when a solution of haematoxylin in pure distilled water is mixed with a very dilute solution of an ordinary iron salt, such as ferric chloride, a deep blue-black coloration is immediately produced.

If, instead of a solution of an ordinary iron salt, a solution of highly colloidal iron or dialysed iron hydroxide be mixed with the stain, a brownish colour is produced, while if organic iron is used, no change in colour takes place. This fact has been explained by Roscoe and Schorlemmer as due to the organic iron compounds having all their possible valences united to carbon atoms.

This staining as a test for iron is quite different from the ordinary use of haematoxylin as a nuclear stain, in which case a mordant is always used, either preceding the haematoxylin, as for example, the iron-alum mordant in Heidenhain's haematoxylin-iron method, or simultaneously, as in the use of the haem-alum stain.

In Macallum's process no mordant whatever is used, but only a solution of haematoxylin in pure distilled water. This gives the colour change only where

a mordant, *e.g.* iron, is naturally present in the tissues. In using it not only must even minutest traces of iron in the water and other fluids be avoided, but also all traces of alkali and acid, since these interfere with the delicacy of the reaction—alkali gives a rose-red colour with the haematoxylin, and acid inhibits the development of the blue-black colour when the amount of iron is small.

To make the staining solution 0·3 gram of pure haematoxylin is dissolved in 50 c.c. of twice distilled water, and kept in Jena glass flasks, since the alkali dissolved out from ordinary glass rapidly turns the solution pink.

Plant tissues, after being prepared, were stained for 12 hours in this solution, while sections of animal tissues were stained for 48 hours.

This was found to be by far the most satisfactory method for the detection of the inorganic iron, and was used in all the animal tissues, and nearly all the plant tissues investigated. Sections so stained can readily be made permanent by dehydrating with alcohol, and mounting in Canada balsam.

Experimental Results.

Section A. Plant Tissues.

In examining plant tissues, it was found that taken as a whole they gave the iron reactions more rapidly and intensely than animal tissues.

In the various grades of plant life it was found that the lower organisms gave a more intense reaction than those higher in the scale. The staining occurred in three different places—in the nuclei, in the chloroplasts, and in large masses scattered throughout the cytoplasm. Leaves of the wall-flower did not show any definite iron staining. Leaves of ordinary water-cress, gave a very definite reaction for iron; large masses, stained bluish-black, were scattered irregularly throughout the leaves, and many of the chloroplasts were also stained.

Descending lower in the scale, sections of various types of sea-weed were examined. The majority of these showed definite iron staining, particularly in the nuclei and the chloroplasts. The staining in the nuclei was specially well marked, while in some of the sections there were also large deeply-stained masses irregularly situated in the cytoplasm.

The reactions for inorganic iron were most marked with the lower plants, such as unicellular green plants, isolated or bound together in delicate alga threads. The algae observed were Vaucheria, Spirogyra Ulva, and Ulothrix. The nuclei and chloroplasts took on a very deep bluish-black colour very readily; the rest of the cytoplasm was usually unstained, but sometimes patches giving the brown colour of colloidal iron were observed.

Diatoms readily took on a dark blue colour, the central part of the cell more intensely than the rest.

Section B. Animal Tissues.

In this series the maximum amount of staining occurred in the lower organisms, *e.g.* Crustacean and Molluscan types, while the Mammalia gave only slight reactions.

In adult *Mammalian tissues* taken from guinea-pigs, sections of the liver showed only very slight iron-staining. This occurred chiefly in the nuclei of the cells in the form of minute, darkly-stained granules, readily visible with the $\frac{1}{12}''$ objective.

In sections of the stomach, the epithelial cytoplasm was unstained, but the granules in the nuclei, specially the nuclei of the muscle fibres, were well stained.

The spleen shows very definite staining, in the form of small irregular granules scattered in large numbers throughout the tissue; granules in the nuclei are also stained.

Sections of the kidney, testicle, and ovary show only the granular staining in the nuclei, except that in the kidney there is also some slight staining of the glomeruli.

In all the mammalian tissues the staining is more marked in the cells which immediately surround the blood vessels.

In *foetal tissues*, taken from the guinea-pig, the reaction was in most of them much more readily obtained than in adult tissues.

In the foetal liver, both the granules scattered throughout the cytoplasm and those in the nuclei were more deeply stained than in the adult liver.

In the foetal kidney the staining was similar to that found in the adult.

The spleen gave very definite reactions, both the granules in the nuclei and the granules irregularly scattered throughout the cytoplasm being more deeply stained than in adult tissues.

Placental tissues, also taken from the guinea-pig, showed very definite staining in patches in the chorionic villi, while most of the nuclei were also stained.

In *human blood smears* only a slight blue coloration of the red cells took place, not sufficient to indicate the presence of inorganic iron.

In *avian tissues*, taken from the sparrow, inorganic iron could be very readily detected. In the liver the sections showed large numbers of darkly stained granules scattered throughout the cytoplasm and also in the nuclei. The cytoplasm (when using a high magnification) also shows very minute smaller granules which give an intense reaction for inorganic iron.

In the kidney, the glomeruli and the lumina of the convoluted tubules are well stained.

In the spleen, no staining could be detected, and in the ovary staining was confined to the nuclei.

These avian tissues on the whole, specially the liver, gave a much more definite reaction for iron than did the mammalian tissues.

In *amphibian tissues*, taken from the frog, the reactions were still more marked. In the liver, there were large numbers of small darkly-stained granules, scattered throughout the cytoplasm, in addition to the well-known pigment masses which occur in unstained sections of frog's liver.

In the kidney, the chief staining took place in the granules of the nuclei.

The spleen showed small darkly-stained granules scattered throughout the cytoplasm, in addition to large masses of pigment like those found in the liver.

The cytoplasm of the red corpuscles was stained a brownish colour, suggesting the presence of colloidal iron, while inorganic iron was present in small granules in the nuclei. The nuclei of the leucocytes were stained dark blue.

In tissues taken from *gold-fish* the hepato-pancreas treated with haematoxylin showed two or three deeply-stained small round granules in the cytoplasm of each cell—these granules were independent of the nucleus and were found in unstained sections to be highly refractile and colourless. They were also strongly stained by the ferrocyanide method. Sections of the kidney and ovary showed similar granules, but not to the same degree. In its blood films the red corpuscles were stained brown, presumably owing to the presence of colloidal iron; the rest of the film was unstained.

In the *cray-fish* the hepato-pancreas gave a very well-marked reaction, the granules in the nuclei being more deeply stained than in any other animal tissue examined. There were also well-stained granules scattered throughout the cytoplasm.

In the *gonidia*, the ova showed a well-marked reaction.

The staining was more intense in cray-fish tissues generally than in any of the higher animals studied.

In the *oyster*, the hepato-pancreas and the gills showed numerous granules scattered through the cytoplasm, and well-stained nuclei.

In the *earth-worm*, the granules in the cell nuclei were extremely well shown and the cuticle was also well stained.

In *hydra* the chloroplasts in many of the cells show a well-marked bluish stain.

In *swimming prawns* and "*plankton*," the swimmerettes are stained uniformly deep blue.

In *sagitta*, the body and tail are also stained deep blue.

Conclusions.

1. Inorganic iron is more widely distributed throughout animal and vegetable tissues than is generally realised.

2. The lower plants and animals give the reaction for inorganic iron much more strongly than do the higher ones.

3. Granules containing inorganic iron are present in almost all the nuclei of plants and animals.

4. Aquatic animals, either marine or fresh water, contain more inorganic iron than those living on land.

5. Foetal tissues contain more inorganic iron than do adult tissues.

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