however, during the formation of the exospore a number of nuclei make their appearance in the oospore around a central oil-globule. These observations do not agree with those of Fisch. According to this observer all the nuclei of the oogonium fuse together to form the nucleus of the oosphere. According to Dangeard the central nucleus of Fisch is nothing more than an oil-globule, and, so far as my observations go, I agree with him. Soon after the separation of the oosphere a quantity of oil begins to accumulate in or near the centre. This is stained deeply by hæmatoxylin or picro-nigrosin, and might be easily mistaken for a nucleus. Its oily nature may be determined, according to Dangeard, by soaking the sections for some time in chloroform, when it disappears and a vacuole is left. The oil-globule gradually increases in size until the exosporium is fully formed. It then takes up about one-third of the diameter of the cavity of the oospore.

The disappearance of the nuclei of the oosphere during the earlier stages of its development is probably only apparent, some change taking place, of the nature of which we are not yet cognisant. It is probable that these nuclei are included in the oosphere at the time of its separation from the gonoplasm.

The problem of fertilisation is an important one, but is difficult to settle. At an early stage the antheridium contains numerous nuclei which pass over at a later stage into the fertilising tube of the antheridium, but whether they pass into the oospore is a question which I have not been able to settle.

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7. *On the Affinity of Nuclein for Iron and other Substances.*

By Professor G. Gilson, *of Louvain.*

An iron-holding nuclein was discovered some years ago by Bunge[1] in the yolk of the hen's egg, and another by Zaleski[2] in the liver cells of various animals. Grounded on these two observations, as well as on some personal researches, Macallum, of Toronto, was led to the generalisation that the nuclein of every cell contains iron as a necessary constituent of itself. I know also that R. Schneider, in 1888, presented to the Physiological Society of Berlin microscopical preparations showing the reaction of iron in the nucleus; but I have not been able to find out where he published an account of his work.

Macallum, like Schneider,[3] tried to detect iron in the nucleus itself by microchemical means. He succeeded in that by keeping cells under the action of ammonium sulphide, in a warm oven, a rather long time—two or three weeks. Recently, however, he declared he has arrived at the same results by a much easier process;[4] but, as far as I know, he has not, up to this time, published his new method. The biological importance of Macallum's conclusion is too obvious to need any further explanation. But on the other hand, as long as there remains the slightest doubt about the fact itself, all kinds of theoretical considerations on the subject would be of no use to positive science. I took upon myself to verify Macallum's observations, and I found very soon that the question is by far more difficult than it might appear.

I also succeeded in detecting iron in the nucleinic elements or chromatosomes of the nucleus, not only by the action of ammonium sulphide alone, but by various other means.

Generally speaking, an intact nucleus gives no reaction with the usual reagents of iron compounds. But I remarked, on the contrary, that when nuclein has been recently submitted to the action of *various chemical agents*, it very clearly gives evidence of the presence of the metal. Amongst the numerous substances I

[1] Bunge, 'Ueber die Assimilation des Eisens,' *Zeitsch. f. Phys. Chemie*, vol. ix. p. 49.

[2] Zaleski, 'Studien über die Leber,' *ibid.*, vol. x. p. 453.

[3] Macallum does not quote Schneider's observations, and seems not to be aware of their existence.

[4] Macallum, *Proc. Roy. Soc.*, April 30, 1891.

tried, sulphuric acid and sulphurous anhydride gave the best results, though many other agents, especially saline solutions, produce the same effect.

When nuclei, previously steeped for a certain time in these liquids, are treated with ammonium sulphide their chromatic elements take a greenish-black colour, while the action of an acidulated solution of potassium ferricyanide gives them an intense blue colour, caused by the formation of Turnbull's blue.

These reactions are strictly limited to the chromatic filaments or chromatosomes of the nucleus, the protoplasm remaining absolutely colourless. In presence of such a perspicuous reaction, there cannot remain the slightest doubt about the presence of iron in the nucleinic elements actually under observation.

But a question now arises: Is Macallum right when he contends that 'the chromatin of every cell, animal or vegetable, is an iron compound'?—that is to say, a regular chemical combination with fixed proportions.

I have no peremptory objection to oppose to Macallum's conclusion. But, though his experiments seem to have been carefully carried out, I must confess I am not entirely satisfied with the accuracy of his generalisation, and cannot help thinking that the union of nuclein with iron might be a rather accidental combination taking place after death only, and similar to that which it effects with many other substances, especially colouring matters.

This suggestion is not a mere supposition; it proceeds from some observations I have made lately.

I was able to ascertain that dead nuclein has a very strong affinity for iron compounds. The nuclei of freshly extracted cells, when steeped in a solution of sulphate of iron, $FeSO_4$, even as weak as 1 for 2,000 parts of water, take with ammonium sulphide or potassium ferricyanide a much more intense colouration than they did before, when simply treated with sulphuric acid. A consequence of that observation on dead nuclein is, that it is extremely difficult to ascertain whether living nuclein really contains iron, or whether it only absorbs it, after it has been killed, out of the blood or other surrounding liquids, or even out of the reagents themselves if they are not absolutely free from iron. Biologists ought therefore to test their reagents very carefully before using them, and also to take great care to avoid the contact of the slightest trace of organic or inorganic iron with the cells. And that is not an easy task at all, for everyone knows that this metal spoils everything, and is everywhere throughout nature.

For my part, though my reagents were as pure as possible, I am not certain at all, as far as the present, that the iron made visible in my preparations really belonged to the living nuclein, and had not been absorbed after death only. Macallum did not experimentally ascertain this affinity of nuclein for iron compounds. I know, however, that he used to treat his objects with an acidulated alcoholic mixture, called Bunge's fluid, a liquid which is supposed to take out, after ten hours of action, all the organic and inorganic iron, excepting that combined with nuclein. But I have observed that Bunge's liquid does not take away the iron artificially combined with dead nuclein even after six days.

I observed also that various iron compounds are attracted by nuclein, and that its affinity is stronger for the ferrosum radical than for the ferricum. But iron is not the only metal which nuclein can absorb and retain. I succeeded in fixing in it manganese, nickel, and even copper, which all gave in the nucleus only their usual reactions. Nickel is almost as strongly attracted as iron itself is. Molybdenum also is retained by nuclein, and this last observation induces me to be cautious about another method of micro-chemical technic recently published. Dr. Lilienfeld, just a month ago, announced he had discovered a method of detecting phosphorus in the nucleus, with the aid of ammonium molybdate and pyrogallol. I suspect that the yellow-brown colouration he regards as characteristic of the phosphorus is caused by the accumulation of ammonium molybdate in the nuclein; the brownish colouration that ammonium molybdate itself gives with pyrogallol is darker in the nucleus, not because nuclein contains phosphorus, but because it retains more of the ammonium molybdate than the protoplasm does.

I may add also that other substances found in the cell have a similar attraction for iron. Macallum pointed out that amyloid substances contain iron, and I

have observed that the silk of certain insects (chironomus, for instance) seems to possess a stronger affinity for this metal than nuclein itself.

To sum up briefly the present state of the question, it is now certain that *dead* nuclein, as well as other substances found in the cell, have a very strong affinity for various compounds of iron and of other metals, or even negative chemical bodies. Thus the difficult question arises, whether the presence of iron in the nucleinic element *during life* is constant and normal, and whether this metal is necessary for the chemical activity of the nucleus.

I hope these remarks will induce other biologists to undertake similar researches on a question that seems to require more than a single man's activity; and that is the reason why I resolved to publish my results in their present incomplete form.

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8. *A Method of Staining Chromatin by Chemical Means.*

By Professor G. Gilson, *of Louvain.*

The fact that metallic compounds are easily fixed in nuclein suggested to me a peculiar method of staining nuclei that, perhaps, might be of some use for special histological and cytological researches. It consists either in keeping cells, previously hardened, in the metallic solution during a certain time, or in adding this latter directly to the usual fixing liquids. Then, after the solution has been thoroughly washed out, the objects are put into ammonium sulphide or potassium ferricyanide, or into both, and washed again.

I succeeded by this method in staining the chromatic elements of many animal and vegetable cells, after the action of picro-sulphuric acid, Perenyi's fluid, corrosive sublimate, especially the acid solution used in my laboratory, and others. But I did not succeed up to the present, after using Flemming's fluid or other chromic liquids.

The colouration is always characteristic, provided the object has been sufficiently washed before the reagent is applied.

I am still studying the best method to get a good dark colouration. Satisfactory results, however, have been obtained with the following solution, as well for fixing and hardening the cell as for staining the nuclei:—

	c.c.
Iron sulphate, 10 per cent. aq.	10
Nitrate of nickel, 50 per cent. aq.	10
Alcohol, 90 per cent., or, better, aldehyde saturated with sulphurous anhydride	10
Sulphuric acid	2
Alcohol, 90 per cent.	40
	72

After some hours' steeping in this fluid, objects are washed with weak alcohol, then with water, and put into ammonium sulphide for a few minutes. After another washing with alcohol and with water they are steeped for about half an hour in a weak solution of potassic ferricyanide acidulated with hydrochloric acid and washed again.

Each of the substances composing the solution, except alcohol, has the effect of making nuclei colourable with ammonium sulphide or with potassium ferricyanide; but I noticed that better results are obtained when they are mixed together in the above-mentioned proportions.

The action of potassium ferricyanide is much quicker and more intense when it succeeds that of ammonium sulphide. This latter does not harm in the slightest way the cells previously treated with the mixed solution or with the usual fixing liquids, though it seems better, at all events, not to use it too concentrated.

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9. *A proposed Reform in Botanical Nomenclature.*

By James Britten.