News, Notes and Queries

CELL CHEMISTRY IN MIESCHER'S DAY

1969 MARKS the centenary of the discovery of nuclein (nucleoprotein) by the Swiss physiologist Friedrich Johann Miescher (1844–1895). He established 'nucleins' as a new class of compounds at a time when proteins were widely regarded both as the most important of all organic substances and as capable of forming compounds with phosphorus and sulphur. Within a surprisingly short time nucleins came to be regarded as the substance of heredity, but it is notorious that the establishment in 1909 of a simple formulation of their structure—the tetranucleotide—turned the clock back so that proteins reoccupied the position of prominence which they had not enjoyed since Miescher's discovery. In order to describe the state of cell chemistry in the 1860s it is necessary to sketch in the early development of organic and histochemistry.

Organic chemistry started in a crude and eclectic manner with analyses of whole organs, extracts and products—flour, milk, cheese, eggs, meat—only much later was the chemistry of tissues and cells made the object of researches. Sugars and fats were quickly recognized from both the animal and plant kingdoms, but proteins, until 1838 termed albuminous substances, were thought to be formed only in the former. The early discovery of gluten in wheat grains by the Italian chemist I.B. Beccari (1682–1766),[1] who recognized its affinity with the albuminous substances of animals, seems not to have attracted much attention, and it was the French chemists Fourcroy in 1791 and Vauquelin in 1802, who first made chemists aware of the significance of albuminous substances in the plant kingdom.

Nonetheless, the chemistry of plants and animals followed largely separate paths which the introduction of the cell theory, a chemical theory of cell formation at that, failed to unite. The dominant feature in plants was the carbohydrate 'membrane' (cell wall) which was nowhere to be found in the cells of animals. In the latter one had to do with glycogen, albumin, fibrin and the like; in plants cellulose, maltose, sucrose and starch were the dominant substances. Indeed the 1830s and 1840s could well be dubbed the starch era of botany. Every textbook worthy of the name included a goodly section on the shapes of the grains, their behaviour towards iodine, acid and so forth. Schleiden with characteristic asperity and marked chauvinism remarked in 1840:

> Starch flour has been so studied of late that an extensive literature has arisen on it. We have not got much further; however we Germans have had the triumph of seeing the French, after making many easily avoidable detours, on account of superficiality in the work, reach the position already attained by Fritsche ten years before, and now the whole phantasy dreamed up out of the air in the manner of Raspail is being thrown into the literary lumber room.[2]

François Vincent Raspail (1794–1878), despite his 'fantastic' theories, had demonstrated by what later became known as the xanthoproteic, Liebermann and aldehyde tests the presence of albuminous matter in the cytoplasm (he called it sap) of plant cells;[3] but he failed to underline the importance of this discovery and shortly after his first publications on the subject he was imprisoned for his political activities. Thus it

https://doi.org/10.1017/S0025727300014800 Published online by Cambridge University Press

came about that two of his countrymen, Anselm Payen (1795–1871) and Brisseau Mirbel (1776–1854), who both knew Raspail personally, jealously guarded their 'priority' over the demonstration of albuminous compounds in young plant tissues. In 1842 they deposited a manuscript in a sealed packet with the Académie des Sciences which was read in 1843. This together with Payen's detailed memoirs, which cover work carried out between 1834 and 1846[4] on the chemical composition of plants, put proteins at the centre of life and of the metabolic pathways in the cell, for Payen revealed by comparative analyses and staining the existence of a positive correlation between nitrogen content and the activity and youth of tissues. The young tissues of root tips, buds and shoots, and especially the root hair cells, were deeply stained by the tannin solution and the mercury protonitrate solution, and their nitrogen content was high. When it was pointed out that such young cells have proportionately large nuclei the inference was made that the nuclei are rich in protein.

This opinion, it was thought, would account for the reaction of nucleus and cytoplasm to iodine, the former turning dark brown, the latter yellow to brownish-yellow. Carmine, however, was absorbed only by the nucleus which became deep red. Clearly the proteins could not be identical in both parts of the cell. The English microscopist Lionel S. Beale (1828–1906) realized that the behaviour of the nuclei to carmine in ammoniacal solution was due to its acidic property, but he was alone in drawing this inference and Fritz Miescher, whose introduction to cell chemistry came through organic chemistry, was doubtless unaware of Beale's very popular lectures to the Royal College of Physicians, which had been translated into German in 1862, and contains this perceptive remark.[5] Beale's *Lectures* are one long polemic, albeit interwoven with much useful information for the student of animal tissues, for his hypothesis of germinal and formed material. In the hands of an original man like Ernst Haeckel they proved seminal, but on the part of the author they constituted a tiresome piece of iconoclasm with which he hoped to demolish the rigid morphological cell dogma of the day. He wanted to supplant the latter by a vitalistic theory in which those structures showing the vital phenomenon of motion (cyclosis, production of pseudopodia or movement of the whole cell) were termed *germinal materials* since Beale concluded that only in them is the vital force active, organizing inert pabulum and constructing out of it inert *formed material.* The nuclei and nucleoli he defined as germinal matter, but in the resting nucleus the material was quiescent, a reserve of germinal matter. On the other hand his criterion of vitality compelled him to regard the whole wandering leucocyte as germinal matter. Since he failed to locate nuclei in circulating red blood corpuscles he designated these as formed material, derived from the younger nucleated cell with germinal matter. Finding that an ammoniacal solution of carmine stained the nuclei and nucleoli red after passing through the cytoplasm unchanged he regarded this stain as a test for germinal matter. Clearly the nucleated cells of the frog red blood corpuscles contained germinal matter since they took up carmine. It was from these centres (nuclei and nucleoli) that the activity of the vital force acted in a centripetal fashion, dividing up to form fresh centres from which new cells arose.

Two years before Miescher went to Tübingen to study the chemistry of the cell, Ernst Haeckel's famous book, *Generelle Morphologie der Organismen* (1866), appeared,

in which the chemistry and function of the nucleus and the cytoplasm were discussed in some detail. The influence of Beale's functional distinction between parts of the cell is evident in Haeckel's conclusion that the nucleus is responsible for the transmission of hereditary qualities, while the cytoplasm is concerned with the adaptation of the cell to its surroundings.[6] Both, he stated, contain protein, but that in the nucleus is distinguished from that in the cytoplasm by small physico-chemical differences. His description of these compounds is representative of that time and deserves quoting:

> The class of albuminous substances, albuminates or proteins, to which all modifications of the active living plasma belong, is above all other substances remarkable on account of its numerous and very important characteristics. Its extremely complicated composition out of five or six kinds of atoms (C, H, O, N, S and frequently P) put it above all other organic compounds.[7]

It is to Haeckel's lasting credit that he opened the minds of biologists to the possibility that the nucleus might have an important role at a time when the protoplasm dominated the field. Miescher's uncle, Wilhelm His (1811–1887) also realized that little was known about the roles of nucleus and cytoplasm, but he was averse to much that the cytologists stood for and directed his nephew to approach the subject from the chemical angle. Miescher never forsook his uncle's camp. Both men resented the cytologists' introduction of fresh levels of organization in open defiance of the biophysicists' programme of reducing physiology to the molecular level. As a medical student in Basle, Miescher had been nourished on the biophysicists' dream and his imagination fired by it. His subsequent exposure to such an ardent biophysicist as Ludwig in Leipzig must have kept alive in him the aims of this group of biologists.

When Miescher left Basle for Tübingen in the spring of 1868, however, he was content to immerse himself completely in the techniques of the organic chemist at the hands of the eminent Adolph Strecker (1822–1871) before embarking on the research topic he had brought with him from Basle—the chemical characterization of nucleus and cytoplasm. This investigation was carried out in the famous castle laboratory of Felix Hoppe-Seyler (1825–1895), who was the author of the standard textbook on techniques in organic chemistry. This book included a section on the extraction of organic compounds from organs and tissues. He described current attempts at obtaining definable constituents from such cells as spermatozoa and pus corpuscles. The latter, he said, contain various fats, cholesterol, protagon (a phosphatide) and albuminous compounds which have been but little studied. On treating with 10% sodium chloride solution.

> . . . one gets a cloudy, thick, slimey mass; if this is filtered a feebly opalescent fluid passes through, and this, treated with distilled water, gives a precipitate with the properties of myosin. It may well appear doubtful that pus is particularly rich in phosphorus as some authors allege, on account of its phosphorus necrosis.[8]

Miescher had carried out preliminary experiments in Basle on the chemical constituents of the white blood corpuscles from lymph glands. This choice of material was dictated by the fact that such cells were regarded as undifferentiated, and their

motility, described so beautifully by Recklinghausen in 1863, showed unmistakably to the cytologist of the day that here one had to do with genuine protoplasm. Wilhelm His, being himself an expert on lymph glands, may well have played a part in this choice. But such glands proved hard to come by in sufficient quantity in Tübingen, so Miescher turned to pus cells which, with the aid of sodium sulphate solution and much patience and hard labour, he succeeded in extracting from the discarded surgical bandages of the local hospital, supplied through the kindness of Drs. Bever and Koch. This was a natural choice, for in medical texts of the day pus cells figured among the most readily accessible cells for study. But Miescher, who required large quantities of pus, had to resort to a messy and unhygienic source—old bandages. The next step—extraction of the nuclei from the cells proved difficult; more often than not the usual solvents produced a slimey mass, just as Hoppe-Seyler had described. It was known that dilute HCl causes breakdown and solution of the protoplasm, so Miescher tried the effect of this acid. After several weeks exposure to a 1/1000 solution followed by shaking the residue with a water/ether mixture he was able to collect a fine powder, which the microscope showed to consist of somewhat shrunken nuclei, with clear contours and nucleoli.

Hoppe-Seyler so much admired this elegant technique that he inserted a description of it in the third edition of his *Handbuch* (1870) before he had allowed Miescher's work to be published. But for Miescher the yield was too low and the technique too lengthy. Determined to obtain more information about the substance of the nuclei than its solubility characteristics he searched for another technique of extraction and lighted upon pepsin digestion, which had recently been used with success by Wilhelm Kühne (1837–1900). In his *Lehrbuch* of 1868 Kühne described how this enzyme caused the greater part of the cells to dissolve, 'leaving behind small crumbs and very shrunken nuclei'.[9] Applied to pus corpuscles it supplied Miescher with a powder identical with that yielded by his treatment of the cells with acid, this time more rapidly and in sufficient quantities to permit an elementary analysis. This showed the presence of 14% nitrogen and 5% phosphorus. This P content was too low to refer to nucleic acid. Clearly he was dealing with the nucleoprotein, and since histones do not contain sulphur, the presence of 2% sulphur was an indication of the impurity of the product. At the same time, Miescher's acid extract when dissolved in weak alkali gave a yellowish solution which must have been the sodium salt of DNA. Even this powder, however, must have been fairly impure since it was insoluble in water.

Payen's 1843 analyses of nuclear-rich tissues had shown their high nitrogen content, but he had not estimated phosphorus. Where spermatozoa had been analysed the phosphorus found was assumed to belong to the phosphatides. Hoppe-Seyler, we have seen, was not impressed by reports of phosphorus in pus corpuscles. In 1868 there had been a dispute in his laboratory over the existence or non-existence of protagon, a phosphatide extracted from brain tissue. Small wonder, then, that Hoppe-Seyler refused to accept Miescher's claim to have found a new class of compounds which he termed 'nucleins'. Hoppe was fearful lest the pepsin digestion had yielded peptones which in some way combined with phosphatides to give a product with a higher N/P ratio than in the only well-authenticated phosphatic organic compound known, namely lecithin. The story is well known how Hoppe, together with two of his

research students, set out to verify and extend Miescher's find, and all ended happily with the publication of Miescher's paper of 1869 together with three supporting papers in 1871.[10]

By the time his work had been verified, Miescher had spent a year under Karl Ludwig in Leipzig and returned to Basle. There, with his uncle he turned his chemical skills to the examination of the structures in the fowl and the salmon egg. He maintained that the most reliable criteria for distinguishing nuclear structures was the presence of nuclein. This he thought he had found in the white yolk platelets of the fowl egg, so he supported his uncle, in opposition to all the cytologists of the day, in asserting that these were nuclei.[11] Unfortunately the chemical characterization was at this stage too imprecise to serve as sole criterion and it was not until 1880 that Albrecht Kossel was able to distinguish between true nucleins and what he called the 'paranucleins' of egg yolk and milk. Over half a century later the paranucleins were shown to contain serine phosphate esters. These give the xanthoproteic reaction and their N/P ratio is close to that of nucleoprotein.[12]

While Lionel Beale struck out on his own because he was opposed to the physicochemical doctrine of life and the biophysicists' approach in particular, Fritz Miescher, a disciple of the biophysicists, relied solely on chemistry because he mistrusted knowledge founded on staining techniques, the chemistry of which was unknown, and because he resented the cytologists' introduction of morphological entities lacking chemical characterization. In his now famous paper on pus cells he mentions only one staining test, that of iodine. The swelling of the nuclei and the fading of the iodine coloration on addition of weak sodium bicarbonate, he said, was well known to histologists as a property of the nuclei. He seems not to have used carmine solution, the only distinctive nuclear stain then available, but sought negative evidence from protein tests. Unfortunately the xanthoproteic, biuret and tannin reactions were not all negative, which encouraged Miescher to regard nuclein as closely related to the proteins. On the basis of its behaviour towards iodine Hoppe-Seyler thought nuclein must be related to amyloid substances (starch).

It is to Miescher's lasting credit that he persisted in a task for which current techniques were inadequate, and that he perceived the importance of his find. But he made it in a cytological vacuum over the nature and function of the nucleus, when chromosomes were unknown and nuclei were thought of as centres of growth, the nucleoli being the foci of such centres. During the 1880s all this was swept away, and the chromosome cycle established in its place. Thus it came about that the meeting point of the cytology and histochemistry of the nucleus was established by the Russian botanist E. Zacharius in 1881.[13] Using pepsin to digest away the cytoplasm he showed that the structures remaining were soluble in alkalis, a solution of iodine in KI in the presence of dilute HCl coloured them brown. Thus far he was only following Miescher, but then he examined the effect of pepsin on the dividing nucleus and found that the stainable nuclear elements of Strasburger's studies (chromosomes) remained when all else had been digested away. Concentrated HCl had the reverse effect, and dilute sodium phosphate solution caused the chromosomes to swell leaving the nuclear spindle unchanged.

In the ten years between the appearance of Miescher's paper on pus cells and

Zacharius' paper on pollen cells and red blood corpuscles, nuclein was pushed to the forefront as a candidate for the role of hereditary substance. Here Miescher's demonstration of the high nuclein content of salmon spermatozoa in 1874 proved a decisive factor.[14] Although he shrunk from identifying the process of hereditary transmission with any one substance Oskar Hertwig, Julius von Sachs and Zacharius were prepared to take the plunge. But many cytologists were not. Flemming, the discoverer of mitosis, was not satisfied that enough was known about nuclein to call it the substance of the chromosomes, so he introduced the word 'chromatin'. Modern research has justified Flemming's caution, but has also established the truth of the assertion that nuclein is the hereditary material.

REFERENCES

1. Beach, E. F., *J. Hist. Med. All. Sci.*, 1961, **16**, 354.
2. Schleiden, M. J., *Flora*, 1840, **23**, 2, 745.
3. Raspail, F. V., *Ann. Sci. d'Obs.*, 1829, **1**, 72.
4. Payen, A., and Mirbel, B., *Ann. Sci. nat. Bot.*, 1843, **19**, 197. Payen, A., *Mém. prés. div. Sav. Acad. Sci. Inst. Fr.*, 1843, **8**, 163, and 1846, **9**, 143.
5. Beale, L. S., *The Structure of the Simple Tissues of the Human Body*, London, 1861, p. 16.
6. Haeckel, E., *Generelle Morphologie der Organismen*, 2 vols., Berlin, 1866, Bd. i, p. 288.
7. Ibid, p. 276.
8. Hoppe-Seyler, E. F. I., *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 2nd ed., Berlin, 1865, p. 363.
9. Kühne, W., *Lehrbuch der physiologischen Chemie*, Leipzig, 1868, pp. 49–50.
10. Miescher, J. F., *Med.-chem. Untersuchungen*, 1871, **4**, 441–460.
11. Ibid., pp. 502–9.
12. Professor J. S. Fruton personal communication; also see Fruton and Simmonds, *General Biochemistry*, New York, John Wiley, 2nd ed., 1958, pp. 55–56.
13. Zacharius, E., *Bot. Ztg.*, 1881, **39**, 169.
14. Miescher, J. F., *Verh. naturf. Ges. Basel*, 1878, **6**, 138–208. This part of Bd. **6** appeared in 1874.

ROBERT OLBY