The Cell-theory: a Restatement, History, and Critique

Part V. The Multiplication of Nuclei

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SUMMARY

1. The belief that nuclei arose by *exogeny*, without relation to pre-existent nuclei, was due mostly to Schleiden (1838). Kölliker (1843) supposed that new nuclei arose by *endogeny* within pre-existent nuclei.

2. Other early theories of the origin of nuclei contained a considerable element of truth. Many early workers thought that the ordinary nuclei of many-celled plants and animals multiplied by *division* (Bagge, 1841; Nägeli, 1844; von Baer, 1846), or by the *disappearance* of the old nucleus and its immediate *replacement* by two new ones (Nägeli, 1841; Reichert, 1846).

3. The history of the discovery of *mitosis* falls into three parts.

In the first (1842–70), chromosomes were seen accidentally from time to time, but no special attention was paid to them (? Nägeli, 1842; Reichert, 1847).

In the second (1871–8), metaphases and anaphases were repeatedly seen, placed in their right sequence, and recognized as normal stages in nuclear multiplication (Russow, 1872; Schneider, 1873; Bütschli, 1875; Strasburger, 1875).

In the third (1878 onwards), the main features of prophase and telophase were described and it was shown that the chromosomes replicated themselves by longitudinal division (Flemming, 1878–82). The separateness of the chromosomes in prophase and the constancy of their number were discovered (Rabl, 1885).

4. These researches proved that in ordinary mitosis the nucleus neither disappears completely nor divides. In certain Protozoa, mitotic division of the nucleus is a reality.

5. The indirect origin of cells, through the intermediacy of syncytia, was established by the work of Nägeli (1844), Rathke (1844), Kölliker (1844), and Leuckart (1858).

6. There is nearly always a cellular phase at some stage or other of the life-history of organisms, even when all the somatic tissues are syncytial. Certain Zygomycetes provide an exception.

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INTRODUCTION

WE are still concerned with Proposition III in the formulation of the cell-theory adopted in this series of papers: that is to say, with the proposition that *cells always arise, directly or indirectly, from pre-existent cells, usually by binary fission.* In Part IV of the series (1953) we traced the history of the discovery that cells multiply by division. As we saw in Part II (1949), cells are defined by their possession of *protoplasm* and a *nucleus*. In cell-division the protoplasm divides (Part IV, 1953). It remains to trace here the history of our knowledge of the way in which nuclei multiply. We shall concern ourselves with the discovery that nuclei are genetically related to pre-existent nuclei, and with the gradual revelation of the real nature of that relationship. The history of the discovery of the rest of the process of mitosis (the behaviour of the centrioles, asters, &c.) is irrelevant to our purpose and will not be considered. The remainder of the paper will be concerned with the indirect origin of cells from cells, by the production of syncytia and the subsequent formation of cells in or from these.

The discussion of Proposition III will be completed in Part VI of the series, which will deal with the continuity of cells from generation to generation.

A few words about the purpose of this series of papers would, I believe, be appreciated by some readers.

The cell-theory has been subjected to powerful attack. As a result, its validity has been questioned in zoological textbooks. I decided to study the evidence against it very carefully, in the original papers. Having done this, and examined the whole subject more widely, I reached the conclusion that the theory withstood the attacks. I then decided to try to persuade others of its validity. I found that I could only develop my argument and make myself understood by a historical treatment, with critical comments from the modern point of view. In many fields of science we must recognize an embryology of ideas: our modern outlook can only be fully grasped and assessed if we understand the causes that make us think as we do. This applies particularly to the cell-theory. Though I have great respect for the history of science, yet my main purpose in this series of papers has not been to write history, but to use a mainly historical method to establish what I believe to be an important truth about living organisms.

EARLY THEORIES OF NUCLEAR MULTIPLICATION

Before telling the history of the discovery of cell-division, it was necessary, in Part IV of this series of papers, to describe the wholly erroneous theories that were for long entertained about the process by which cells multiply. Our understanding of the multiplication of nuclei has come in a different way. Some of the early theories were wrong, but they were not wholly wrong; and considerable interest attaches to them in so far as they led towards the discovery of mitosis. First, however, it is necessary to eliminate a theory that con-

tained no element of truth. This was the theory that nuclei arise exogenously in what Schwann (1839, pp. 45 and 207–12) called a *Cytoblastem*, without any relation to pre-existing nuclei.

Exogeny

Valentin (1835, p. 194) appears to have been the first person to make a suggestion as to the origin of nuclei. He claimed that in the chorioid coat of the eye, nuclei arise by a process of precipitation. He confuses his remarks by calling the nuclei *Pigmentbläschen*, though they are colourless; the globules of pigment appear subsequently round them.

The theory that nuclei in general arise exogenously was due to Schleiden (1838, pp. 145–6). His ideas have already been given in detail in Part IV of this series of papers (1953, p. 416), and need only be briefly mentioned here. It may be remembered that in his view, a nucleolus appeared without any relation to a pre-existing nucleus, and the nucleus or *Cytoblast* was formed round this by deposition of a granular coagulum. This nucleus then produced a cell round itself. In his first paper (1838a), Schwann accepted Schleiden's scheme and applied it to animals. It was unfortunate that the first ideas about the multiplication of nuclei were completely wrong, yet supported by two famous investigators.

Henle (1841, pp. 153–4) was evidently affected by these beliefs. He shows a cartilage-cell (his plate V, fig. 6) with a nucleus containing a nucleolus at one end and a body resembling a nucleolus at the other. He suggests rather tentatively that a nucleus had just formed round one of the nucleoli. Kölliker at one time thought that nucleoli might appear spontaneously in certain cases, by the crystallization of granules in a homogeneous fluid, and that nuclei were subsequently formed round them (1844, pp. 143–4 and 150); but, as we shall see (p. 452), he supposed that nucleoli ordinarily multiplied by division within nuclei.

Nägeli at one time allowed that nuclei might originate without any relation to pre-existent nuclei (1846, see especially pp. 62–63).

During the eighteen-forties, the belief that new nuclei arose in some sort of connexion with pre-existing ones became quite general, but the older view still lingered on. One cannot fail to regret that Remak, who had done so much to elucidate the multiplication of cells, eventually retracted a little from the position he had taken up and began to equivocate. He came to believe that new nuclei might in certain cases originate independently of pre-existing ones. He thought that when small blood-vessels were developing in the cutis of the frog, new nuclei appeared that were not related to the embryonic nuclei; he remarked also that the stellate cells (presumably fibroblasts) of connective tissue developed without any known connexion with the cells of the embryo. He also thought that new cells originated in diseased tissues without any participation of pre-existing nuclei (1862, p. 282).

Remak was not the only distinguished investigator to continue to hold such views. Weismann (1863a), in his account of the development of the egg of

Chironomus, says that nuclei 'appear' (*erscheinen*) at the same moment over the whole of the blastoderm, which then separates itself off round each of them and thus forms uninucleate cells. Lankester (1875, pp. 38–41) thought that in the development of *Loligo*, the 'autoplasts' (nuclei of the yolk-epithelium) were of the same nature as the nuclei of the blastomeres, but for the most part of independent origin. (The actual origin of the yolk-epithelium of cephalopods was finally revealed in the next decade by Vialleton (1888).)

Endogeny

Kölliker appears to have been the only person who claimed that new nuclei arise endogenously within old. Mainly as a result of his studies of the embryology of nematodes and of the frog, he reached the conclusion that the nucleolus lengthens, constricts, and divides; a new nucleus then forms endogenously round each of the two nucleoli thus produced, within the membrane of the mother-nucleus (Kölliker, 1843; 1844, pp. 143–4, 150). All this he described in the puzzling nomenclature that has already been described (Part IV, p. 418). Later he summed up his opinion in very clear language. 'Nuclei and cells multiply by endogenous procreation', he wrote; 'nucleoli by division' (1845, p. 96).

The theory of endogeny bore little relation to the actual events of nuclear multiplication, but at least it involved a genetic relationship between old and new nuclei. The two theories to be discussed next came nearer to reality. Indeed, each of them revealed a considerable part of the truth.

Division

Even at the present day we often read in biological textbooks of nuclear division, though in fact, of course, typical nuclei do not, in any intelligible sense, divide. Exceptions to this are provided by certain Protozoa (see below, p. 474), and also by the polyenergid nuclei of certain other members of the same phylum (see Baker, 1948*a*); but the latter are better regarded as representing aggregations of many small nuclei. It is true that the nuclei of certain tissue-cells of higher animals have been supposed to multiply by 'amitosis'; but in fact it seems unlikely that such a rough-and-ready method could divide the gene-complex accurately enough to produce viable cells (though it might suffice in a syncytium). It is more likely that in these cases disguised mitoses occur, without regular, easily recognizable metaphases and anaphases.

Ehrenberg (1838) appears to have been the first to witness the multiplication of a typical nucleus. He saw clearly the nucleus of the protomonad flagellate *Monas vivipara*, but regarded it as the testis. He remarks (pp. 9–10) that it divides when the animal divides, and he gives a figure (his plate I, fig. IV*a*) professing to show a stage in this process.

Barry (1841, *a*, *b*, and *c*) thought that nuclei multiply by division, or rather fragmentation, and that the fragments become new *cells*. This view was accepted by Goodsir (1845, p. 2). Barry worked chiefly with the red blood-corpuscles of various vertebrates. His observations on this subject, however,

are so unsatisfactory that they cannot be regarded as having contributed to knowledge. In an earlier paper (1839) there are some passages (on p. 361) that suggest at first glance that he witnessed nuclear multiplication in early mammalian embryos, but this is not so.

Bagge (1841) reported nuclear division in the early embryo of '*Ascaris nigrovenosa*' (= *Rhabdias bufonis*). He recognized that the duplication of the nucleus preceded that of the cell. His terminology is unfortunately misleading: he calls nuclei *cellulae* and cells *vitelli partes* or *globulae*. His illustration, here reproduced as fig. 1, is probably the earliest attempt to represent consecutive stages in the process of nuclear multiplication.
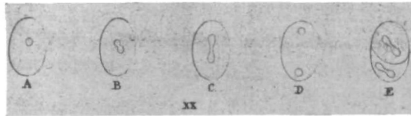

FIG. 1. Stages in supposed nuclear division in *Rhabdias bufonis*. Bagge, 1841 (fig. xx).

Remak (1841) studied blood-formation in the late chick-embryo. He noted that in dividing blood-cells the two nuclei were joined together by a stalk-like process (probably the remains of the spindle). Later (1845) he tried to follow the way in which nuclei multiply in the developing striated muscle of the frog tadpole. He was at first hesitant, remarking cautiously, 'I am not able to assert that the formation of new nuclei proceeds always from those already present, though several observations suggest this.' Further study of the same object convinced him that the new nuclei arise by division of the old (1855, p. 154), and he illustrates what he takes to be a division stage (his plate XI, fig. 5). Neither the text nor the figure gives any details of the process. Writing about nuclear multiplication in general, he admits (p. 174) that the process has not been elucidated with certainty, but claims that it clearly begins with a constriction. He leaves it undecided whether the nuclear membrane dissolves.

Valentin briefly described and figured a stage in the division or *Spaltung* of the nucleus of a cell in the membrane covering the auricles of the frog's heart (1842, p. 629 and plate VII, fig. 95 *bis*, *a* and *b*).

Breuer (1844, p. 31) and his associate, Günsburg (1848, pp. 361–2), claimed that in regenerating mammalian skin, nuclei multiplied by division or fragmentation (*sejunctione*). These authors may perhaps have been looking at the nuclei of polymorphs. Günsburg thought that the nucleus generally fell into as many pieces as there were nucleoli.

Nägeli (1844) described the division of the nucleus of a germinating spore of *Padina* (Phaeophyceae). His figure showing the two nuclei, supposed to have been produced by division of the old nucleus before the cell had divided, was reproduced on p. 433 of Part IV of this series of papers (1953). Nägeli subsequently came to regard division as the usual method of nuclear multiplication in plants (1846, pp. 68–69), though not, as we shall see (p. 457), the only method.

With von Baer we enter a new phase. His account of the process of nuclear multiplication was far fuller and more accurate than anything that had been published previously.

Perhaps because he published (in German) in a Russian journal, perhaps because his paper takes the shape of an informal, chatty letter from the seaside, von Baer's contribution (1846) to our understanding of the multiplication of nuclei has not received the credit it deserves. During his stay at Trieste he artificially fertilized the eggs of '*Echinus*' (*Paracentrotus*) *lividus* and watched the process of cleavage. In his description we can follow what was actually happening. It is very helpful to place beside his description a set of figures of the cleavage of the same animal made much later by Hertwig (1876). These figures are here reproduced in fig. 2, as an illustration of von Baer's paper. They show what can be seen in life. It will be noticed that Hertwig did not see the chromosomes (though he saw them clearly enough when he fixed and stained the embryo). Von Baer's great merit is that he gave a realistic description of what can be seen of mitosis when the chromosomes themselves are not seen.

Von Baer correctly identifies the nucleus (*Kern*) of the unfertilized egg. He tells us that on fertilization it sinks more deeply into the egg, and its limits become more difficult to see. He does not recognize the participation of the nucleus of the spermatozoon in the process, but thinks that the egg nucleus alone is the progenitor of those of the embryo. 'After some period of rest', he writes, 'this nucleus, up till now spherical, lengthens rather quickly by sprouting, as it were, at both sides; both ends swell, but the middle becomes thinner and soon divides completely, so that two comet-shaped nuclei with their tails lie opposite one another. Then, very quickly, the tail-shaped appendages pull themselves back into the spherical or vesicular masses, and one has two nuclei. . . . Before the division the original nucleus had already increased in volume; during the division this happens still more, so that each of the two new nuclei has apparently the size of the original one.' The egg now divides. 'Soon afterwards each of the two nuclei now begins to sprout out in the same way, and, dividing in the middle, changes into two new nuclei, round which the yolkmass then likewise divides, and the whole egg resolves itself into four masses adhering to one another. . . . Quite similarly there follows the division of the quadrants, and indeed in such a way that the direction of the new sprouts stands at right angles to the immediately preceding ones. So it goes on with new divisions, for a nucleus forms itself in advance for each portion of the yolk by division of one that was produced earlier.' He remarks here that a pellicle is formed round the nucleus each time after a period of rest. 'Up to the division into 32 yolk-bodies, when the process is occurring quite regularly, I have been able to watch the division continuously.' The appearance is now for a time less clear. 'But still, when the embryo has left the egg-membranes and is moving itself by means of cilia, each granule or histogenetic element (vulgarly called a "cell") has a very evident nucleus, and they all appear to be derived from the original nucleus of the egg.' Movement of the larva now
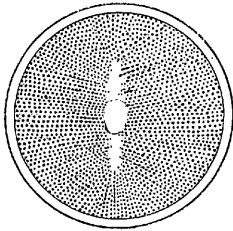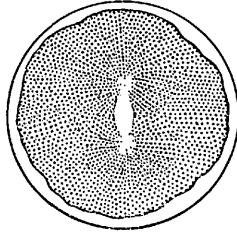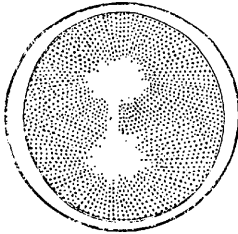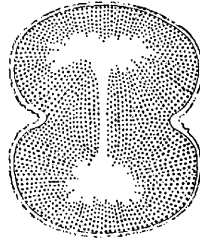
Fig. 15.

Fig. 16.
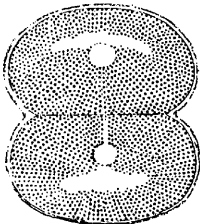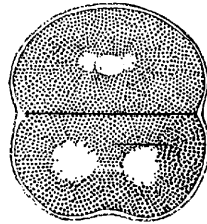
Fig. 17.

Fig. 18.

Fig. 19.

Fig. 20.

FIG. 2. Stages in nuclear replication in the embryo of *Paracentrotus lividus,* to illustrate von Baer's description. Hertwig, 1876 (plate XII).

makes observation difficult. 'But I have reason for the belief that the perma-
nent tissue-constituents also arise from the original one by quite similar divi-
sions. According to this, the divisions of the yolk would only be the beginnings
of the histogenetic separation that progresses continuously up to the final
formation of the animal' (1846, cols. 237–40).

Von Baer obviously saw the spindle and regarded it as a nucleus that had
elongated in preparation for division. This outlook has not quite left us even
today. One finds in textbooks statements to the effect that the spindle is
formed from the nuclear sap. In fact, the nuclear membrane disappears during
late prophase in most organisms other than certain Protozoa, and the nuclear
sap then merges indistinguishably with the ground cytoplasm. Even if one dis-
regards the fact that part of the spindle is often clearly formed in the cytoplasm
while the nuclear membrane is still intact, it is still unjustifiable to derive
the spindle exclusively from the nucleus. Even if this were not so, it would
still be wrong to speak of nuclear multiplication by division; for in those
cases in which the spindle-remnant survives to be divided across at cell-
division, the products of its division are not incorporated in the new nuclei.

Virchow (1857) considered that nuclei ordinarily divide by a process of
constriction. Gegenbaur (1858, pp. 9–10) saw something of nuclear multi-
plication in the cleavage of the egg of *Sagitta*. He admits that the details of
the process escaped him, but he remarks that he saw a stage in which the
nucleus was drawn out to a great length, and many were provided with con-
strictions. He presumably saw spindles and took them for elongated nuclei.
He supposed that the actual division of the nucleus must take place very
quickly.

Schultze (1861, p. 11) followed up his famous definition of a cell (see
Part III of this series of papers (1952), p. 165) with a generalization on nuclear
multiplication in the very next sentence. 'The nucleus and also the proto-
plasm', he wrote, 'are division-products of the same components of another
cell.'

In his study of the development of *Musca vomitoria*, Weismann (1863*b*,
p. 162) announced that each of the four pole-cells (primary germ-cells) divides
into two, with simultaneous division of their nuclei.

As we shall see, a number of botanists had adopted the view that the nucleus
disappears at cell-division and is somehow replaced by two new ones. Hanstein
(1870) devoted a paper to the refutation of this belief. He worked chiefly with
the parenchyma of various flowering plants. He satisfied himself that the
nucleus did not disappear. He claimed that it was constricted by a delicate but
optically perceptible halving-boundary (*Halbirungsgrenze*), and that when this
process was complete, the two halves of the nucleus moved apart to opposite
poles and a new cell-wall was formed between them (pp. 230–1).

Disappearance and replacement

The supporters of the theory of nuclear division performed a useful service
by calling attention to the fact that two new nuclei are somehow derived from

one old one, but they overlooked a rather obvious part of the usual process—
the disappearance of the nuclear membrane and nucleolus. While some investi-
gators were claiming that nuclei divided, others insisted that on the contrary
a nucleus *disappears* and is *replaced* by two new ones. Each side in the contro-
versy had seized upon one aspect of the truth.

If a nucleus completely disappeared and was then replaced by two new
ones, the latter could be regarded as having arisen exogenously; but it seems
desirable to draw a distinction between the origin of a new nucleus without
any relation to a pre-existent nucleus, and the disappearance of one nucleus and
its replacement by two new ones.

From his studies of pollen-formation, Nägeli (1841) concluded that when
the mother-cell is about to divide, the cytoblast (nucleus) is absorbed. A new
cytoblast then appears in each of two granular areas in the cytoplasm. Mem-
branes form in such a way as to enclose each of the granular areas. The whole
process is then repeated, with disappearance and replacement of the cytoblast.
Thus four cells are formed, each with its nucleus. Alternatively, the four cells
with their nuclei may be formed simultaneously after the disappearance of the
nucleus of the mother-cell. Later, in a general account of pollen- and spore-
formation, Nägeli repeats this general scheme, with the added complication
that the original nucleus of the mother-cell, lying against the cell-wall, dis-
appears and is replaced by a central nucleus, which in turn disappears and is
replaced by four new ones or by two which are each subsequently replaced by
two (1844, pp. 83–84 (p. 84 is accidentally numbered 48)).

As we have already seen (p. 453), Nägeli regarded division as the usual
method by which the nuclei of plants multiply; but he retained his belief that
in particular cases there is absorption and replacement (1846, p. 70).

Hofmeister thought it certain that the nucleus of the pollen mother-cell of
Tradescantia underwent dissolution (*Auflösung*) and replacement by two new
nuclei (1848*b*, col. 651).

Meanwhile, similar results were being obtained with animals. Reichert
studied the egg and embryo of the nematode *Strongylus auricularis* (1846,
pp. 201 and 255–6). He described the disappearance or *Hinschwinden* of the
germinal vesicle and the mixture of its contents with the substance of the rest
of the egg. A new nucleus was formed, but this again disappeared. A new one
was formed in each of the first two blastomeres; these again disappeared
before the next division. So the process went on. The nucleus underwent
Hinschwinden before each division, the newly-formed cells contained no
nucleus, and finally a new nucleus appeared in each. Reichert followed the
repetition of this process up to the stage at which the form of the little worm
had become visible. He illustrated his findings by careful drawings (his
plate IX). Similarly, Krohn described the disappearance of the nucleus at
each cleavage division in the ascidian *Phallusia*, and the reappearance of a
nucleus in each newly-formed blastomere (1852, pp. 314–15).

Much later than this, at a time when chromosomes had often been seen, it
was still supposed that the germinal vesicle of the primary oocyte did in fact

wholly disappear when the polar bodies were about to be given off. This is perhaps not surprising in view of the large size of the vesicle in relation to that of the chromosomes. Thus van Beneden described the complete disappearance of the germinal vesicle in the rabbit (1875, p. 692). He remarks that at this stage the egg is what Haeckel called a *Cytode*; that is to say, a lump of cytoplasm not containing a nucleus (see Haeckel, 1866, pp. 273–4).

Auerbach also used the term *Cytode* for the cell in which the nucleus has disappeared before cell-division (1876, p. 258). He thought that the substance of the nucleus intermingled with the cytoplasm and dissolved in it. For this reason he termed the stage of mitosis at which the nucleus becomes no longer visible *die karyolytische Figur* (p. 222).

Mitosis

It is a fact that there is genetic continuity between old nuclei and new, but nuclei do not ordinarily multiply by division. It is a fact that the nuclear membrane and nucleolus disappear at cell-division, yet the whole of the nucleus does not vanish. The truth could only be established when the erroneous parts of each theory had been eliminated, and when the remainder had been integrated by the discovery of something important that had been overlooked by both—the chromosomes.

If we study the old papers in which the early descriptions of chromosomes appear, it seems at first almost impossible to give an intelligible exposition of the way in which our modern knowledge was achieved. Yet there is an evolutionary story to be told, for there were in fact *stages* in the history, more evident, no doubt, to us who look back than to those who lived through the events.

In the first stage there were mere accidental records of bodies that we can now recognize as chromosomes. In the next, metaphases and anaphases were repeatedly described, and came to be regarded as usual stages in the process of nuclear duplication. It was understood that the anaphase was subsequent to the metaphase. In the third and last stage, the prophase and telophase were carefully described, and the real nature of the genetic relationship between one nucleus and the two that succeed it was disclosed through the genius of Flemming.

The first period (1842–70)

In a general account of the changes of form of the nucleus, Henle (1841, pp. 193–4) makes some remarks that suggest strongly that he saw some of the stages of mitosis. He says that nuclei often become oval and then more elongated, and then change into thin striations. The nucleoli disappear and the nucleus then becomes decomposed into a row of little dots (*Pünktchen*). He mentions that nuclei are sometimes connected by threads. If he had left it at this, we should probably have believed that the striations represented the spindle and the *Pünktchen* the chromosomes; but he illustrates what he saw

by drawings, and a study of these suggests that he was not looking at stages in
cell-division after all.

Valentin (1842, pp. 630–1) says that after treatment with acids, nuclei are
sometimes seen in the act of division, 'with granular accessory appendage'.
One can only guess whether the granules were chromosomes. No details are
given that could guide us.

Nägeli is the first person of whom we can say that he probably saw chromo-
somes. In his account of the formation of pollen in *Lilium tigrinum*, he
describes how the cytoblast of the mother-cell vanishes and is replaced by
a variable number of small cytoblasts, which are transitory and in their turn
disappear (1842, pp. 11–12). He figures a cell containing seven of them (see
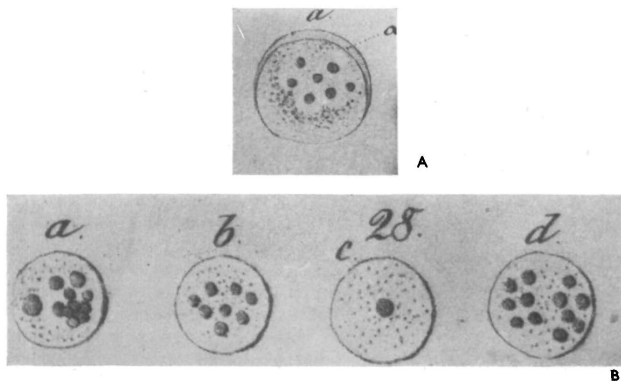

FIG. 3. These are probably the earliest illustrations of chromosomes. A, 'transitory cytoblasts'
in the pollen mother-cell of *Lilium tigrinum*. B, the same, in pollen mother-cells of *Tradescantia*.
Nägeli, 1842 (plates I, fig. 12d, and II, fig. 28; enlarged from the original figures).

fig. 3, A). When they have disappeared, the contents of the cell divide in two,
and a new cytoblast is formed in each of the products of division. It is possible
that these transitory cytoblasts were chromosomes, though doubt is engen-
dered by the fact that in some cases there were only one or two of them in the
cell. He saw similar appearances in the pollen mother-cell of *Tradescantia* (see
fig. 3, B). It seems likely in this case that the bodies were actually chromosomes.
Three of the cells illustrated contain 11, 8, and 12 such bodies. Nägeli thought
it probable that when these transitory bodies had disappeared, a single, large
cytoblast was formed (in the case of *Tradescantia*), and this divided in two
(p. 13).

Later, in describing what was presumably the division of the pollen mother-
cell of *Amaryllis*, he gives a figure of what he calls the *Kern* but is actually the
spindle (1844, plate II, fig. 24a). He remarks that this body has 'dunkle

körnige Anhänge'. These may have been anaphase chromosome-groups, but the figure is unfortunately on too small a scale to show them.

If Nägeli probably saw chromosomes, then Reichert (1847) certainly did so. He investigated carefully the spermatogenesis of *Strongylus auricularis*, noting that the developmental stages could be followed in time-sequence by passing along the testis from its blind end. After mentioning what were evidently the spermatogonia at the blind end of the tube, he goes on, 'Besides these, vesicles [cells] sometimes occur which contain two nuclei, of exactly the same microscopical constitution, but smaller [than those of the other cells], and also vesicles that are provided with no nuclei at all, but only with separate small granules (*Körnchen*); these vesicles cannot be mistaken for the clear vesicles that are perhaps of artificial origin' (p. 101). Serious artifact is indeed unlikely, for Reichert simply opened the testis at chosen places to let out the contents, and wetted the preparation with saliva or egg-white (p. 99). The *Körnchen* were evidently chromosomes, probably those of spermatogonial mitoses, for he thought that the nucleus actually disappeared altogether in what we should call the meiotic divisions (pp. 110–13).

Hofmeister (1848*a*) had the great merit of realizing that most contemporary cytologists were devoting a disproportionate amount of attention to the cell-wall and neglecting the nucleus. He decided to study nuclear phenomena in the pollen mother-cells of *Tradescantia virginica*. He found that as the cell grew, so did its nucleus and nucleolus; but eventually, at about the same time, the nucleolus and nuclear membrane disappeared. As we have already seen (p. 457), Hofmeister believed in the actual *Auflösung* of the nucleus in *Tradescantia*, and its replacement by two new ones. He thought, however, that when the nucleus had just dissolved, the albuminous material occupying what had been its site was in a particularly coagulable condition. He produced coagulation by the action of either water or tincture of iodine. Either of these agents produced separate *Klumpen* in the cell (cols. 427–30). These objects, which he supposed to be artificial coagulates, were in fact chromosomes (see fig. 4). He considered them to be of the same nature as the transitory cytoblasts of Nägeli.
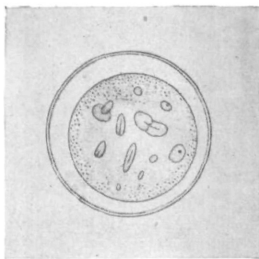

FIG. 4. The chromosomes in a pollen mother-cell of *Tradescantia virginica*, after treatment with tincture of iodine. Hofmeister, 1848 (plate IV, fig. 10*b*).

Nineteen years later Hofmeister still retained the same opinion. As a result of studies of the formation of pollen in various phanerogams and of spores in vascular cryptogams, he concluded that at the stage of disappearance of the nuclear membrane, the substance of the nucleus is easily coagulated as a little clot of strongly refractive substance, or else in the form of numerous, much smaller objects (1867, p. 81). The latter were undoubtedly chromosomes. He

mentions that in spore-formation in *Equisetum* they are situated in the equator of the cell; in *Psilotum* they arrange themselves in a horizontal plate. Fig. 5 is a reproduction of Hofmeister's illustration of a meiotic metaphase in *Psilotum 'triquetum'* (= *nudum*). Fifty-four bodies, presumably chromosome-pairs, can be counted in this figure. The haploid chromosome number in this species is probably in fact 52 (see Manton, 1950, p. 239). Hofmeister's figure shows a remarkable resemblance to the same stage in *P. flaccidum*, as illustrated by Manton (her fig. 236; 52–54 chromosome-pairs). One cannot fail to be struck by such an accurate chromosome-count at this early date.

FIG. 5. The chromosomes in a spore mother-cell of *Psilotum nudum.* Hofmeister, 1867 (fig. 16, *e*).
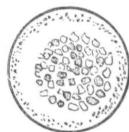
Hofmeister once again attributed the appearance of chromosomes to artificial coagulation by water. In the circumstances it is surprising that he should have given such an accurate representation of their number.

Virchow (1857, p. 90) saw a cell with what he calls a branched nucleus among dividing cells in a cancerous lymph-gland. His figure suggests that he may possibly have seen a metaphase (his plate I, fig. 14).

Henle (1866) saw what were apparently pachytene stages (his figs. 263, 268) and perhaps the metaphase and telophase of the first maturation division (fig. 266) in mammalian spermatogenesis. His figures and descriptions are too vague, however, for certainty to be reached. He used acetic and chromic acids as fixatives (pp. 355–6), and one would expect the meiotic chromosomes to have been visible. Indeed, it is rather strange that there are so few early accounts of chromosomes in testes, for it would only have been necessary to examine the organs of almost any animal during the season of spermatogenesis to see at any rate the chromosomes of the first prophase. Such stages were seen much later by Spengel (1876) in the spermatogenesis of several genera of Gymnophiona. He compared them to Chinese writing (p. 31, see his plate II, fig. 33).

Chromosomes were probably seen by Krause (1870) in the epithelial cells of the surface of the cornea of various mammals. He called them granulated oval corpuscles (*Körperchen*). He thought that the nuclei of the epithelium multiplied by division, but did not claim that the corpuscles were necessarily connected with this process. Subsequently he gave an illustration of these bodies (1876, fig. 8, *f*). The figure seems to represent a late prophase.

The first period of chromosome studies, which started with Nägeli in 1842, ended with Krause's paper of 1870. Up to the latter date the descriptions and figures were vague and unsatisfactory, so that we generally cannot tell exactly what stage of mitosis or meiosis was seen, and we cannot even be certain, in some cases, that chromosomes were seen at all. No attempt had been made as yet to arrange the stages of mitosis in a time-series.

The second period (1871–8)

During the second period discoveries about chromosomes came with a rush. Kowalevski (1871) published the first figure of chromosomes that really

resembles the object. One can tell instantly on looking at his drawing that he saw an anaphase (see fig. 6). His object was a section through the embryo of the lumbriculid worm *'Euaxes'* (= *Rhynchelmis*), at the moment when the first set of micromeres was being given off. He used chromic acid for hardening the egg. He calls the two groups of chromosomes *zwei körnige Anhäufungen* (p. 13). He regarded them as representing division-products of the nucleolus.

Russow (1872) carried our knowledge of chromosomes much further in a study of spore-formation in vascular cryptogams. He found that at the division of the spore mother-cell, a *Stäbchenplatte* was formed (pp. 89, 126). He saw these metaphase plates particularly clearly in ferns and Equisetales. Leaving for a moment the cryptogams that form the main subject of his very long
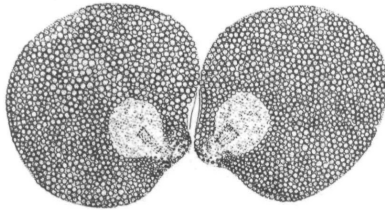

Fig. 6. Anaphase at the formation of the first quartette of micromeres in *Rhynchelmis*. Kowalevski, 1871 (plate IV, fig. 24).

paper, he remarks that he has seen these plates most clearly of all in the pollen mother-cells of *Lilium bulbiferum*. He then proceeds to the first serious attempt ever made at a description of chromosomes. He remarks (p. 90) that in *Lilium* they are short, worm-shaped corpuscles or slightly curved rodlets, colourless, pale, and faintly refractive, scarcely detectably stained by iodine, and almost instantaneously dissolved by alkalis (even at great dilution) and by ammonium carminate; also by chlor-zinc-iodide, without becoming coloured. He noted the same chemical behaviour in the chromosomes of the vascular cryptogams.
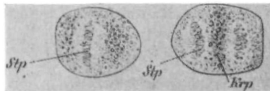

Fig. 7. Metaphase and anaphase in the spore mother-cell of *Ophioglossum vulgatum*. Russow, 1872 (plate VI, figs. 121 and 122).

He distinguished clearly between the *Stäbchenplatte* and the subsequently-formed *Körnerplatte* (cell-plate).

Russow also saw anaphase chromosome-groups (see fig. 7). He called each of them a secondary *Stäbchenplatte*. He did not explain how they arose, and evidently thought they were the metaphase plates of the next division (pp. 127, 204). He noted that when there is a *Stäbchenplatte*, there is never a nucleus, and put forward the possibility that the plate is formed from the nucleus. He noted (p. 90) that when a secondary plate is viewed from the side, it resembles a very granular nucleus. This suggests that he saw a telophase.

Russow strongly denied that the chromosome plate is an artifact, as Hof-

meister had supposed. He saw it in the intact sporangia of *Polypodium* and '*Aspidium*' (= *Dryopteris*).

Schneider's paper of 1873 constitutes a landmark in the history of our knowledge of chromosomes. He followed carefully the cleavage of the egg of the rhabdocoel *Mesostomum ehrenbergii*. By using acetic acid as fixative, he clearly saw the *dicke Stränge* or chromosomes, and noticed that one half of them went to one pole and the other half to the other (see fig. 8). He saw mitosis not only during cleavage, but also during later embryonic stages and in the adult (pp. 113–16 and plate V, fig. 11). He knew that the nucleus had been thought by others to disappear at cell-division, but he considered it probable that the process he had described in *Mesostomum* actually occurred in these cases. It is evident that he thought that amitotic division also took place. Schneider's paper is above all important for its clear seriation of metaphase and anaphase.

Tschistiakoff (1875, *a* and *b*) saw metaphases and illustrated them clearly in spore mother-cells of *Isoetes* (Lycopodinae) and various ferns, and also in pollen mother-cells of *Magnolia* (his plate I, figs. xx and xxiv). He called the chromosome-plate a *Körnchenlamelle* (1875*a*, col. 1). He made the serious mistake of supposing that the two new nuclei were formed at the poles of the spindle while the chromosomes were still on the metaphase plate (1875*b*).

A paper of this period by Ewetsky (1875) is remarkable because it contains the first reasonably good figure of a prophase (see fig. 9). The cell is from the endothelium of Descemet's membrane in the eye, in regeneration following operational damage. Like Russow, Ewetsky called the chromosomes vermiform structures. Like so many others, he thought that the spindle was the nucleus and that it divided, merely enclosing the anaphase chromosome-groups at its ends.

Bütschli (1875) studied polar body formation and cleavage in the nematode *Cucullanus elegans*, a parasite of fresh-water fishes. He saw metaphases and

FIG. 8. The earliest figure showing stages in mitosis in correct sequence. The first cleavage of the egg of *Mesostomum ehrenbergii*. Schneider, 1873 (plate V, fig. 5).

FIG. 9. An early representation of prophase. An endothelial cell of Descemet's membrane. Ewetsky, 1875 (plate V, fig. 7).

anaphases distinctly. Unfortunately his paper is not illustrated. The drawing shown here (fig. 10) is from his publication of the following year. He gives
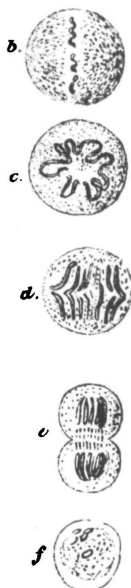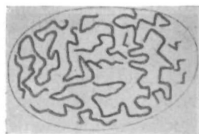
a particularly clear account of the occurrences at the first cleavage (1875, pp. 211–12). He says that the nucleus becomes invisible and a longitudinally striated, spindle-shaped body appears. Each fibre is swollen at the equator of the spindle into a *Korn* or *Körnchen*, and if one looks from the end of the spindle one sees a ring of granules (*Körnchenkreis*). The error of supposing that chromosomes were swollen regions of spindle-fibres was hard to eradicate in subsequent years. It may be remarked that Bütschli's name for the group of chromosomes at metaphase was a much more realistic one than the term
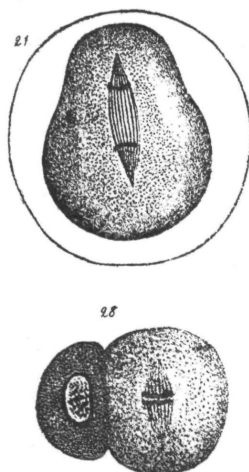

Fig. 10. Typical illustrations of mitosis by Bütschli. Anaphase of the first cleavage and meta-phase of the second in *Cucullanus elegans*. Bütschli, 1876 (plate III, figs. 21 and 28).

Platte, which still survives; for there is no real resemblance to a plate unless the chromosomes happen to be very small, very numerous, and very close together. From the single ring of granules now arise two rings, and these move apart. Bütschli uses the expressive term *Auseinanderrücken der Körnchenkreise* for the anaphase. Although he used acetic acid as a fixative in the investigation, it seems possible that he watched the anaphase in life. He noticed that the spindle and chromosomes disappeared and a new nucleus was formed at each pole. He gives no details of these occurrences. He showed the negative merit of not regarding the new nuclei as division-products of the spindle.

Bütschli, like others, had already (1873) seen and figured spindles (but not chromosomes) in the micronuclear mitoses of *Paramecium*, and he recognized the same object in *Cucullanus*. In 1873 he had regarded the spindles as seminal vesicles.

In his paper of 1875 Bütschli described the fusion of chromosomal vesicles to form a nucleus, but he did not relate the vesicles to chromosomes. Such

vesicles had already been reported by Remak (1855, p. 139 and plate IX, fig. 14), Lang (1872), and Oellacher (1872, pp. 410–11 and plate XXXIII, figs. 29–36). These investigations, however, did not help towards the elucidation of mitosis.

Strasburger was drawn into the study of mitosis by his interest in the process of fertilization in conifers. He worked particularly with the spruce, *Picea vulgaris*. The first two mitoses after fertilization escaped him, but he studied the multiplication of the four resulting nuclei, which are situated at the end
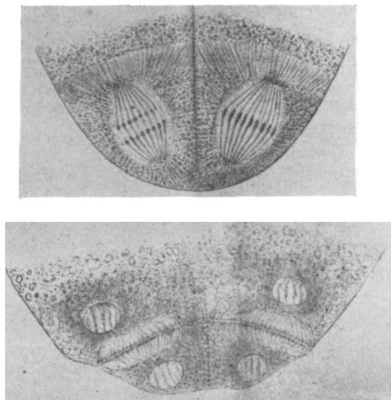

Fig. 11. Typical illustrations of mitosis by Strasburger. Metaphase, anaphase, and telophase in the multiplication of the nuclei at the lower end of the ovum of *Picea vulgaris*. Strasburger, 1875 (plate II, figs. 27 and 30).

of the ovum that is farthest from the micropyle. He used material fixed in alcohol, without staining. He saw metaphases and anaphases clearly (see fig. 11, upper figure). His description (1875, pp. 26–27) shows that he thought the process was one of actual nuclear division. He regarded the spindle, with its equatorial *Platte* of *Stäbchen* (chromosomes), as the nucleus. When the *Platte* separated into two, at the beginning of anaphase, the nucleus had divided. Each daughter-nucleus was formed by the fusion of the chromosomes of one plate with one another and with half the spindle. Strasburger made scarcely any attempt at a description of prophase or telophase, though he did illustrate a telophase (fig. 11, lower figure).

Strasburger realized the necessity of studying the process in life, so as to be sure that alcohol did not produce artificial appearances and also so as to be able to place the stages in their proper sequence with certainty. He found suitable material in *Spirogyra*. He followed the whole process of mitosis in the living alga (1875, pp. 33–46 and plate III), though he was not able to see much of prophase or telophase beyond the disappearance and reappearance of the

nucleolus. He also studied alcohol-preparations, and confirmed their reliability. (Several years later (1879) he found a particularly suitable object for vital studies of mitosis in the staminal hairs of *Tradescantia*, immersed in 1% cane-sugar solution.)

Having found close resemblance between the processes of mitosis in such widely different plants, Strasburger now investigated it in many other diverse forms, and saw similar metaphases and anaphases over and over again. The cells he studied all had short chromosomes, and it is probable that this fact prevented a more complete understanding of what really happens in mitosis.

Strasburger was not content to study plants only. He knew of Bütschli's work on *Cucullanus*, and got into touch with him. Bütschli provided him with unpublished figures of mitosis in *Cucullanus* and of meiosis in *Blatta*, and Strasburger recognized the similarity to what he had seen in plants. Strasburger himself studied mitosis in mammalian cartilage (1875, pp. 186–9) and especially in the cleavage of the ascidian, *Phallusia mamillata* (pp. 189–97).

Strasburger brought the whole of his work together in his justly famous book, *Ueber Zellbildung und Zelltheilung* (1875), in which he included (with full acknowledgement) some of Bütschli's unpublished figures. This book was by far the most complete account of cell-division available at the time, and served to show the universality of mitosis as the ordinary process of nuclear multiplication.

At the time, the writings of Bütschli and Strasburger attracted far more attention than those of the Russian cytologists and Schneider. A number of authors were quick to recognize, in their own research-material, the descriptions given by the two former workers. Van Beneden was one of the first to come under their spell (1875). He studied the process of nuclear duplication in the ectoderm of the rabbit embryo, after fixation with osmium tetroxide and staining generally with picrocarmine (a favourite combination with early students of chromosomes). He recognized the separation of the equatorial plate of refringent globules or *bâtonnets* into two *disques nucléaires*, and the movement of these apart from one another at anaphase. He noted correctly that the new nuclei were formed from the disks, which swelled up at the expense of the surrounding cytoplasm and became less and less easily stainable. Later, in the course of the work that resulted in the foundation of the group Mesozoa, van Beneden saw mitosis in the cleavage of the cell that gives rise to the infusiform embryo of *Dicyemella* (1876, pp. 48–52; plate I, fig. 28; plate III, figs. 2, 4, 11).

Mayzel (1875), also influenced by Bütschli and Strasburger, saw various stages of mitosis in the regenerating corneal epithelium of the frog, including a metaphase with radially-arranged chromosomes (p. 851).

The rarity of Strasburger's first edition reflects the publisher's underestimation of the interest of this new line of research. A new edition was quickly produced (1876), with advice from Bütschli in correspondence and conversation. In the same year Bütschli produced an immense paper (1876), profusely

illustrated with figures of mitosis in the cleavage of a leech, of *Cucullanus* (see fig. 10), and of *Limnaea*, of meiosis in the testis of *Blatta*, and of nuclear changes in the conjugation of various ciliates. It is strange to note his pre-occupation with side-views of metaphases and anaphases. Prophases he scarcely noted. He shows one, however, in *Cucullanus* (his plate III, fig. 20) and suggests that it may represent a preliminary stage (p. 226). If only he had studied metaphases more often in polar view, he might have made important discoveries about the constancy of chromosome number. He still describes the equatorial plate as consisting of the thickened parts of the spindle-fibres (p. 219). Telophase still eludes him, but he thinks that new nuclei must arise from the groups of chromosomes at the two ends of the spindle (p. 220).

Balbiani (1876) found an excellent source of mitotic figures in the ovariolar epithelium of the nymph of the grasshopper *Stenobothrus*. He saw the prophase chromosomes as short, unequal rods and followed them through all the stages of mitosis till they fused at telophase to form a mass that became vacuolated; a membrane then appeared round it. He says that each of the *bâtonnets* cuts itself into two halves, but he gives no indication of the direction of the cutting. This short paper is, for its period, a remarkably complete account of mitosis in a single kind of cell.

The study of mitosis was now taken up actively by O. Hertwig, who produced a succession of papers on polar-body formation and cleavage in leeches, heteropods, echinoderms, and frogs (1876, 1877, 1878, *a* and *b*). An exponent of the osmium / carmine technique (and also of others), his careful studies were marred only by a tendency to follow Bütschli in regarding the chromosomes as swellings of the spindle-fibres. These papers were important because they revealed new facts about polar bodies and fertilization rather than because they established new details of the process of mitosis; but they helped to show how widely applicable were the findings of Bütschli, Strasburger, and the rest.

Eberth (1876) saw mitotic figures in the regenerating cornea of the frog and rabbit, and compared them with Strasburger's descriptions. The latter, in an interesting critique of Eberth's findings, discusses the origin of the spindle (1877, p. 522). He denies that it is derived simply from the nucleus. Sometimes there is no distinction between nuclear sap and cytoplasm at the time when the spindle is formed: the two have become continuous with one another. This message from the past deserves attention at the present day.

One cannot better comprehend the deficiencies of knowledge about mitosis at the close of the second period than by studying the third edition of Strasburger's book, which was published a little later (1880). Though the stages of metaphase and anaphase were by this time so familiar, they were not in the least understood. Strasburger still believed that the *Kernplatte* or equatorial 'plate' of chromosomes became divided at metaphase, and that this division was of a hit-or-miss nature (pp. 331–3). He thought that rod-shaped chromosomes ordinarily arranged themselves along the length of the spindle. Division took place in the same way whether the chromosomes were rods or granules.

If any part of a rod or granule was in the equatorial plane at metaphase, that granule or rod was divided across at that place. Those granules or rods that lay nearer one pole of the spindle passed towards that pole without division. Longitudinal division never occurred except in those particular cases in which long chromosomes arranged themselves at metaphase wholly in the equatorial plane. The splitting then occurred at metaphase.

The third period (1878 onwards)

Five years after the publication of Strasburger's third edition the chief facts of mitosis had been established, chiefly by the brilliant researches of Flemming and Rabl.

Strasburger, as we have seen, had already followed mitosis in the living cells of *Spirogyra*. Particular stages in mitosis had been seen in the living cells of animals. Mayzel (1877), for instance, had put various cells of vertebrates in aqueous humor and seen the stages that he had previously (1875) studied in fixed preparations. No one, however, had watched the actual succession of the stages in animal cells. Now, in 1878, three papers were published by investigators who had seen the process of mitosis unroll before their eyes. Schleicher was the first in the field, with a very short account of mitosis in living cartilage-cells of various Amphibia (8 June). Peremeschko, who had read Schleicher's paper, was next (27 July). He studied epithelial cells, fibroblasts, leucocytes, and endothelial cells of blood-vessels in the tails of young newts ('*Triton*' (*Triturus*) *cristatus*). Schleicher's and Peremeschko's studies were of importance in confirming beyond question the order of the most striking events of mitosis. Peremeschko remarked that the threads were sometimes *knäuelförmig* at the beginning of the process; so evidently he saw something of the prophase. Neither of these authors, however, added any important new facts.

Flemming had already (1877) chosen the salamander (*Salamandra maculata*) as his cytological research-material, on account of the large size of the cells and nuclei in this animal. He now started his research on cell-division, and read a paper on the subject at Kiel on the very day (1 August) on which he first saw a copy of Peremeschko's. This paper (1878) was short, in marked contrast to his massive later contributions. It contains more than just the foreshadowings of the important discoveries that were to come. His research-material was again the salamander, especially its larva. He studied the urinary bladder, the epithelium of the skin and gills, cartilage, connective tissue, the endothelium of blood-vessels, and blood-cells. He followed the whole process of mitosis in life and also studied fixed and stained preparations. Flemming was outspoken in his criticism of Strasburger's scheme of mitosis. Here in this paper one finds the first serious attempt at a description of the *Anfangsphasen* or early prophase-stages. Flemming definitely derives the chromosomes (*Fäden*) from the stainable substance visible in the form of a *Gerüst* in the interphase nucleus. He traces the gradual shortening and thickening of the trabeculae of the *Gerüst* to form finally the chromosomes of the metaphase *Stern*. He considers that the disappearing nucleolus supplies material to the thickening chromo-

somes. He notes that the chromosomes are split longitudinally throughout their length in (late) prophase, a discovery of the first importance, but in this paper he does not trace one longitudinal half to each pole in anaphase. He gives no exact account of what happens at metaphase. He sees the chromosomes move apart at anaphase, and describes the changes of telophase as resembling those of prophase in reverse. Thus for the first time the chromatin was followed through from one resting nucleus to the next. As a result of his studies, Flemming denied that the nucleus could be said without qualification to divide.

Flemming's choice of organisms with long chromosomes as his research-material, both in this early work and later, undoubtedly helped him to elucidate the main features of mitosis.

Flemming now began to produce a succession of papers, the main purpose of which was to show the uniformity of the process of mitosis. He denied that amitosis had been proved to exist in the tissues of animals, except possibly in leucocytes. Indirect nuclear division, with *Fadenmeta-morphosen des Kerns*, was the rule (1879a, pp. 21–22). He now reverted to the *Längsspaltung* of the chromosomes. He saw the longitudinal split in both prophase and metaphase (see fig. 12) and suggested tentatively that one longitudinal half of each thread might go to each daughter-nucleus (1879b, p. 384). In the same paper he describes the prophase. He makes the mistake of supposing that the chromosomes are

FIG. 12. The earliest figures showing the longitudinal splitting of chromosomes. A, epithelial cell from the gill of a salamander larva, to show the longitudinal split in some of the chromosomes at prophase. B, endothelial cell in a small blood-vessel of a salamander larva, to show the longitudinal split at metaphase. Flemming, 1879b (plate XVII, figs. 7 and 11).

joined end to end into a continuous *Knäuel*. This he describes as a thin thread, which thickens and eventually breaks across into separate chromosomes. He once more describes the telophase as prophase in reverse, and gives a tabular synopsis of the whole process, arranged to bring out this resemblance (p. 392). He follows the telophase *Knäuel* into the network (*Gerüst*) of the interphase nucleus.

Flemming emphasized that longitudinal splitting occurs constantly in diverse kinds of cells (1880, p. 212). He doubted Strasburger's belief that there were considerable differences between one case and another, and turned to plant material to find whether his scheme applied there also. Removing the coverslips from someone else's preparations of the embryo-sack of *Lilium croceum*, he restained them to his own satisfaction and was able to confirm
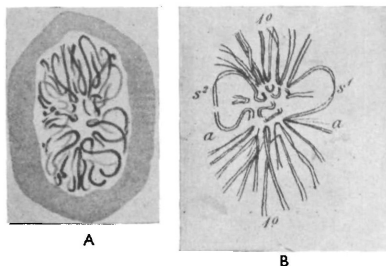
that what he had so often observed in animals occurred also in this case (1882*a*, p. 43). He denied that the chromosomes fuse at metaphase to form a plate.

Flemming's great achievement was his discovery that in mitosis one longi-
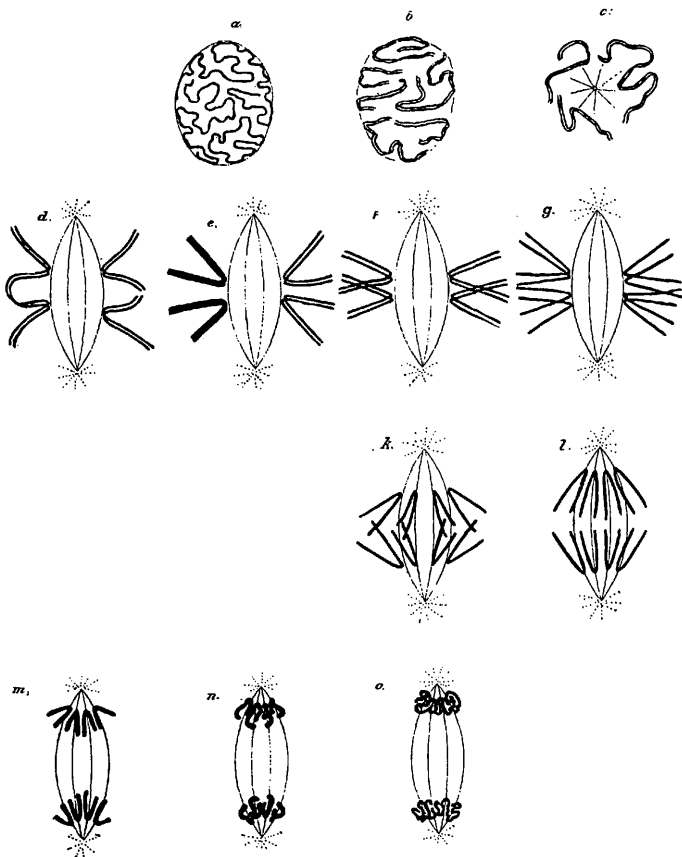
FIG. 13. Diagram of mitosis. Flemming, 1882*b* (plate VIII, fig. 1). (*h* and *i* are here omitted. Flemming used them to illustrate Strasburger's opinions.)

tudinal half of each chromosome goes in each direction, so that each daughter-nucleus is formed from a complete set of longitudinal halves. He brought together his ideas on the basic plan of mitosis in a diagram published in his book, *Zellsubstanz, Kern und Zelltheilung* (1882*b*). The diagram is reproduced

here as fig. 13. The only error of any importance is the joining together of the chromosomes in prophase, end to end, to form a continuous *Knäuel*. The achievement represented by this diagram can be appreciated when it is remembered that only a few years earlier, almost nothing was known of prophase or telophase, and the most essential fact of mitosis—the separation of the chromosomes into two groups of longitudinal halves—was quite unknown.

Rabl's work (1885) was complementary to Flemming's, for it corrected the latter's main error and made good his main deficiency. His material was the epidermis of the floor of the mouth and of the gills of the larva of *Salamandra maculata*, and various organs of *Proteus*. He showed that in the prophase, the chromosomes are not joined end-to-end in a continuous *Knäuel*, but are separate from one another from the first. Further, their number is the same as at metaphase, and this number is always the same (24) in various cells of the two species studied. Rabl carried out his extremely laborious work with the utmost care and skill. One of his figures of a prophase, with separate, numbered chromosomes, is shown here in fig. 14. By 1880, as Boveri (1904, p. 4) remarked, the investigations of Flemming, Strasburger, van Beneden, and others were already leading to the conclusion that the number of chromosomes in the cells of each species was the same or nearly the same, but the paper that decided the issue was Rabl's of 1885. Boveri had great
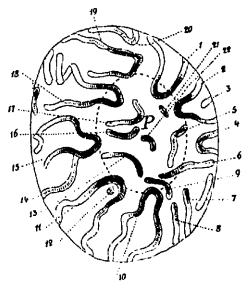

FIG. 14. The chromosomes in a prophase nucleus of an epidermal cell of the larva of *Salamandra maculata*. Twenty-two of the twenty-four chromosomes are shown. Rabl, 1885 (plate XIII, 6th figure).

admiration for the Austrian cytologist. He writes of his 'wonderful perseverance and observational capacity' (1888, p. 5). Rabl studied carefully the early prophase and late telophase stages, and his findings in this field, taken in conjunction with his establishment of the constancy of chromosome number, formed the basis on which Boveri started to build his theory of the individuality or continuity of the chromosomes.

Nomenclature

It is curious that cytologists should have been so slow to suggest international technical terms for the description of mitosis.

Schleicher pointed out the inconvenience of the multiplicity of terms used for the process itself, and suggested *Karyokinesis* (1879, p. 261). Flemming remarked that even in direct nuclear division, there is movement in or at the nucleus, which is what Schleicher's term means. He therefore suggested the substitution of *Karyomitosis* for it, to indicate 'thread-metamorphosis in the nucleus'. Instead of having to say 'nuclear division figures', one might use the short word *Mitosen* (1882*b*, p. 376). It must be regretted that Flemming was so far influenced by his profound study of the long chromosomes of the

salamander as to choose this word, for in very many organisms the metaphase chromosomes cannot by any stretch of the imagination be described as threads.

The terms *Prophase, Metaphase*, and *Anaphase* were coined by Strasburger (1884, pp. 250 and 260). He did not use the terms metaphase and anaphase exactly as they are used today. For him, the metaphase continued until the daughter-chromosomes were quite separate from one another. Today, the anaphase is usually regarded as starting as soon as the spindle-attachments begin suddenly to move towards the poles. There would be little or no difference between the two usages of the word, however, in the case of the short chromosomes with which Strasburger usually worked. His anaphase included the modern anaphase (or late anaphase) and also telophase. His failure to give a separate name to the latter reflects his lack of attention to the final stage of mitosis.

The word *Telophase* was coined by M. Heidenhain ten years later (1894, p. 524). Curiously enough, he did not define it by any change in the chromosomes. His telophase started directly the centrosomes (*Mikrocentren*) left their positions at the poles of the spindle to migrate to their final sites in the new cells.

Interphase was introduced by Lundegårdh (1913, p. 211) to indicate the period between two closely consecutive mitoses. He drew a distinction between interphase and the *Ruhezustand* that follows mitosis when another division will not occur, or will be indefinitely delayed (pp. 213–19). He described differences between nuclei in interphase and those in the resting state.

Very diverse terms were used for chromosomes before that word was at long last introduced. They were called *Fäden, Kernfäden, Schleifen, Stäbchen, stäbchenförmige Körner, Körnchen*, and *chromatische Elemente*. Waldeyer certainly performed a useful service when he introduced *Chromosomen* (1888, p. 27). It would have been difficult to choose a shorter word so applicable to the object named in all its variant forms.

Comment

It is a strange fact that some of the best early workers on chromosomes continued to believe that nuclei ordinarily multiplied by division. Auerbach did not fall into this error. He contributed to the subject a short paper that did not receive the attention it deserved. As we have seen (p. 458), he at first believed in the actual solution of the whole of the nucleus at cell-division. Later, when the participation of chromosomes in the process had been repeatedly described, he justly claimed that there had been an element of truth in his belief. He denied that the spindle is derived solely from the nucleus, and that the main mass of it participates in the formation of the new nuclei; and he protested against the statement that nuclei divide (1876).

To resolve fully the question whether mitosis is nuclear division it would have been necessary to know the structure of the interphase nucleus, but even today this is a subject on which we are very imperfectly informed. Very large chromosomes, especially those of certain Liliaceae and Urodela, have attracted

a great deal of attention from students of mitosis, but there is reason to believe that the nuclei to which they give rise are untypical of nuclei as a whole. As E. B. Wilson remarked (1925, p. 82), the commonest type of nucleus in both plants and animals is the vesicular. Now it would appear, from the important studies of Manton (1935), that the chromosomes occupy a relatively small space in a vesicular nucleus. Independent work by various authors on diverse cells suggests that as a rule the chromosomes of a vesicular nucleus are situated just below the nuclear membrane (see, for example, Ludford, 1954). When each of them has a single heterochromatic segment, it is relatively easy to make sure that these parts of the chromosomes at any rate occupy this situation (Manton, 1935). The form and position of the remainder of the chromosome cannot be defined with certainty, but it appears probable that the euchromatic segments are drawn out into threads in the same part of the nucleus. The nuclear sap occupies a large part of the volume of the nucleus, and the nucleolus (often single) is usually large.

There are all varieties of nucleus between the typical vesicular one just described, through the intermediate types investigated by Chayen and others (1953), to the kind of nucleus that results from the telophase transformation of very large chromosomes. This kind has been carefully studied by Manton (1935) in *Allium ursinum*. It appears that in this species the chromosomes maintain their early telophase positions throughout the interphase, simply swelling up to form almost the whole of the nucleus, so that there is very little room for nuclear sap. The nucleoli are not free to move, and therefore remain separate.

Pollister (1952) has very clearly described and figured two contrasting theories of nuclear structure, but it seems probable that what he describes are really the extreme forms of an object that varies widely in different plants and animals.

Of the regular constituents of the nucleus—membrane, sap, chromosomes, and nucleolus—only the chromosomes can be said to divide in ordinary mitosis. Except in certain Protozoa (see p. 474), the nuclear membrane disappears, and it then becomes impossible to distinguish sap from ground cytoplasm. It is therefore wrong to state definitely that the spindle is formed from the nuclear sap. The spindle is in fact in some cases divided by the formation of a cell-plate, or by the ingrowth of a cleavage-furrow; but there is no evidence either that it is of purely nuclear origin, or that any part of it constitutes a part of the daughter-nucleus; so that even when it is divided, it is not a continuously self-reproducing body. In the present state of knowledge it is not possible to say what parts of the cell, beyond the chromosomes, are concerned in the formation of the daughter-nuclei; but so far as is known, there is ordinarily no direct genetic relationship between the old and the new nuclear membrane, and the same applies to the nuclear sap and nucleolus.

It follows that when *Omnis nucleus e nucleo* was written in imitation of *Omnis cellula e cellula*, the similarity of the two phrases tended to hide an essential difference. The word *e* was being used in two different senses. The

ground cytoplasm of a cell arises from that of a pre-existent one by mere division. Two new nuclei ordinarily arise from a pre-existent one by an entirely different process. No new nucleus will be formed unless a group of anaphase chromosomes is present, and since these chromosomes arose *e* the old nucleus, we may say that the new nucleus to that extent arose *e* the old. But the new nuclear membrane, nuclear sap, and nucleolus did not in any intelligible sense arise *e* the old.

The basic truth of the old Latin aphorisms nevertheless remains. Neither a cell nor a nucleus exists, unless there has been a pre-existing cell and nucleus which gave rise to it either directly (in the case of the cytoplasm) or indirectly (in the case of the nucleus). In Part II of this series of papers (1949) the cell was defined by its possession of protoplasm and nucleus. It is a fundamental part of the cell-theory that these are both, directly or indirectly, self-reproducing parts. The only reservations that must be made about this rule are that protoplasm must originally have evolved from matter that did not possess all its qualities, and that the nucleus, as we know it today in the great majority of plants and animals, must have evolved from a simpler structure in the distant past. The question whether it is legitimate to speak of a nucleus in the Cyanophyceae and Bacteria, and whether there is anything in those groups that throws light on the origin of the definitive nucleus, must be reserved for consideration under Proposition VI.

Although mitosis is not in fact a process of nuclear division in the great majority of plants and animals, including those in which it was first studied, yet mitotic division is a reality in certain Protozoa. Indeed, most of the errors about mitosis, entertained by the early workers on the subject, are not errors at all in the case of many flagellates. It was discovered by Blochmann (1894) that in several species of *Euglena* the nuclear membrane never disappears during mitosis, but simply becomes constricted across. Further, the nucleolus elongates into a rod thickened at each end and finally breaks in the middle, leaving one nucleolus in each of the daughter-nuclei. As Blochmann pointed out, the process is nevertheless mitotic, for chromosomes participate in it. Indeed, their behaviour is nearly normal, except that their arrangement at the stage corresponding to metaphase is less regular than usual. Keuten (1895), who had participated with Blochmann in the original work, was able to show that the chromosomes divide longitudinally and that their division-products separate in the usual way. No definite spindle or centrioles are seen. Alexeieff (1911) showed that this form of mitosis, far from being restricted to *Euglena*, occurs also in protomonads and peridinians, and indeed in certain non-flagellate Protozoa.

The more recent work of several authors, especially Hollande (1942, pp. 111–15), has confirmed the general correctness of Blochmann's and Keuten's findings. In the polymastigine, *Tetramitus*, the process is even more similar to ordinary mitosis, for a spindle is formed and the chromosomes arrange themselves very regularly at metaphase; but here again the whole

affair occurs within a persistent nuclear membrane, and the nucleolus dupli-
cates itself by division (Hollande, 1942, pp. 185–7).

Dangeard (1902) called this form of mitosis *haplomitose*, but it seems ques-
tionable whether it is simpler than ordinary mitosis. Indeed, it is doubtful
whether one can make a general statement that mitosis is usually simpler in
Protozoa than in other organisms, though it is certainly much more diverse.
(See Grassé's admirable review of the strange process of 'pleuromitosis' in cer-
tain Protozoa (1952, pp. 104–16).) The basic facts remain that chromosomes
are concerned in the formation of new nuclei, and that these chromosomes
multiply by longitudinal division.

THE INDIRECT ORIGIN OF CELLS FROM CELLS

History of the discovery

In the formulation of the cell-theory used in the present series of papers,
the third proposition is this: 'Cells always arise, directly or indirectly, from
pre-existing cells, usually by binary fission' (1948, p. 105). It remains to con-
sider the indirect origin of cells from cells; that is to say, the development of
a syncytium from a cell, and then of cells from the syncytium. The existence
of syncytia, but not their development or transformation, has already been
considered in Part III of this series (1952, pp. 177–83).

It was unfortunate that Schleiden (1838) chose a syncytium, the young
endosperm, as his main subject of study when he was trying to find how cells
develop. If he had chosen a tissue in which cells multiply by binary fission, it
is scarcely possible that his ideas on the origin of cells would have been so
erroneous. Through his influence the endosperm became a classical site for
the study of the origin of cells.

Nägeli devoted much attention to cell-formation in endosperm and other
syncytia. One would not suppose, from a study of his writings (1844, 1846),
that binary fission was a more usual method of cellular multiplication. He
performed a very useful service in demonstrating the error of Schleiden's
views. The importance of his discoveries about the origin of cells in syncytia
tends to be blurred for modern readers by the disproportionate emphasis he
placed on his distinction between *freie* and *wandständige Zellenbildung*. In fact,
however, he gave the first adequate account of the origin of cells in syncytia.
He recognized that in endosperm and certain other sites there were numerous
nuclei, not separated by cell-walls. (For his views on the origin of these nuclei,
see above (pp. 451, 453, and 457).) The *Schleim* (protoplasm) lying between
the nuclei now underwent a process of *Individualisierung* round the nuclei, and
a *Membran* (cell-wall) was formed at the surface of each individualized portion
of the *Schleim*. Thus, as many cells were formed as there were nuclei. Some-
times the newly-formed cells were spherical or nearly so, and free from one
another and from the wall of the maternal syncytium; inevitably part of the
syncytial protoplasm failed to be incorporated in the cells. This was *freie
Zellenbildung*. In other cases (*wandständige Zellenbildung*) no protoplasm was

left out of the new cells, for the cell-walls were formed in immediate apposition to one another (or, in the external part of the syncytium, to the maternal cell-wall).

Rathke and Kölliker were the first to describe the origin of cells from syncytia in animals. I have already published a translation of Rathke's words (1952, p. 180). He remarks very tersely that in the embryo of Crustacea, nuclei are formed for the future embryonic cells before the cells themselves originate. He does not mention the mode of formation of the nuclei.

In his study of the development of cephalopods, Kölliker (1844) realized that the *Furchungssegmente* (*blastocones* of Vialleton, 1888) were not separated from the yolk by any distinct boundary. He must have realized, therefore, that the *Embryonalzell* (nucleus) of one blastocone was not separated from that of the next by any membrane; or, to put it in other words, he must have understood that he was dealing with a syncytium. He knew that the nuclei duplicated themselves in the blastocones, and that uninucleate cells (the *Furchungskugeln* or blastomeres) were budded off from their tips. It follows that he described the origin of cells from a syncytium, though this fact is obscured by his strange nomenclature, which I have already explained (1953, p. 418).

Incomplete cleavage is usually called meroblastic, but Remak himself (1852), when he introduced the term *méroblastique*, did not attach exactly this significance to it. For him, an egg was meroblastic if the embryo was formed from only a part of it: if the whole egg clove to convert itself into the embryo, it was holoblastic. It seems uncertain whether the egg of cephalopods would be meroblastic by Remak's definition, despite the fact that the cleavage-furrows do not pass right through it. The meaning of the term that is usual today appears to derive from Nicholson (1870, p. 217), who wrote of the development of the lobster, 'The ovum is "meroblastic", a portion only of the vitellus undergoing segmentation.'

The first really adequate description of the origin of cells from a syncytium in animals was given by Leuckart (1858, pp. 210–11), in his account of the development of *Melophagus* (Diptera Pupipara). He tentatively derived the nuclei of the early embryo from the germinal vesicle of the egg. He remarks that the development of the egg of insects conforms to the usual process of embryonic cell-formation, but 'A difference appears to me to exist here, only in so far as in insects the envelopment of the cell-nuclei with yolk-substance first occurs late, after the number of nuclei has already been considerably increased, while in other cases such an envelopment happens from the beginning, so that the division of the nuclei has then for a consequence, naturally and also constantly, a division of the yolk'. Robin (1862) noticed this method of cell-formation in various culicines; he called it *gemmation* and distinguished it from cleavage. He did not remark, however, on the presence of one nucleus in each of the cells formed by this process. Weismann confirmed Leuckart's findings by his studies of the development of *Chironomus* (1863a, pp. 112–13) and *Musca* (1863b, p. 163). He noticed the rising of the nuclei to the *Keimhautblastem* (blastoderm) in *Musca*, and compared it to the rising of air-bubbles

to the surface of water. When the blastoderm had separated itself off round each of these nuclei, the newly-formed cells multiplied by ordinary binary fission.

Comment

Syncytia that eventually resolve themselves into cells do not constitute an exception to the cell-theory as formulated in this series of papers. Particular parts of the body are often permanently syncytial. Not many groups of organisms are wholly syncytial, even in their somatic tissues. The belief that rotifers provide an example will not withstand critical examination, though many of their organs are wholly or partly syncytial (Martini, 1912; Nachtwey, 1925). The same applies to nematodes.

In the great groups of syncytial plants, the Siphonales, Cladophorales, and Phycomycetes, there is nearly always a periodical reversion to the haplocyte or diplocyte; that is to say, to the *cell* as defined in this series of papers (Part III, 1952). It will be recalled that the gametes of the Siphonales are generally flagellate cells; in *Vaucheria*, in which they are not flagellate, they are nevertheless cells. In the Cladophorales, asexual reproduction is in nearly every case by zoospores in the form of flagellate cells.

The two groups of Phycomycetes differ markedly in their reproductive processes. In the Oomycetes, asexual reproduction is generally by flagellate cells, sexual reproduction by the fusion of uninucleate protoplasmic masses from the antheridium with uninucleate ova, or, in the more primitive forms (Uniflagellatae), generally by the fusion of uninucleate flagellate gametes. In certain Zygomycetes, however, the cellular phase seems genuinely to have been lost. In *Pilobus crystallinus* the sporangiospores are in fact uninucleate, but in some other species each spore is multinucleate, so that asexual reproduction occurs without the intervention of a cellular phase. This applies, for instance, to *Rhizopus nigricans* and *Sporidinia grandis*. Now it is characteristic of sexual reproduction in Zygomycetes that the whole of the syncytial protoplasm of one gametangium fuses with that of another, with subsequent fusion of the nuclei in pairs. The new individual produced by this fusion proceeds to asexual reproduction (without any intercalated cellular phase) by forming multinucleate sporangiospores. The cycle is thus completed without the existence of a cell at any stage of the life-history.

Such forms as *Rhizopus* and *Sporodinia* are of exceptional interest to the student of the cell-theory. Their existence is a disproof that the theory is of universal application. It is to be remembered, however, that we can quote few similar examples in plants, and nothing at all that is even remotely similar in animals, except in certain Ciliophora. A discussion of the latter is reserved for a future paper in this series, which will be devoted to a consideration of the status of the Protozoa from the point of view of the cell-theory. (I have already treated the subject shortly (1948a).)

How has it come about in the course of evolution that the great majority of organisms consist largely of cells or at least are derived from and return to

cells in the course of their life-histories? This is one of the fundamental questions of biology, yet there is not very much that can be said in answer to it.

It is necessary to consider the somatic tissues separately from the gametes, because quite different causes appear to have been at work. In the case of the somatic tissues it is to be noted that a high degree of complexity of structure is never reached in a wholly syncytial organism. The repeatedly-quoted case of *Caulerpa* (Siphonales) is misleading, for the parts that superficially resemble the leaf, stem, and root of higher forms do not attain to anything approaching the degree of differentiation that their external aspect would suggest. It seems that organisms can more easily achieve differentiation in a cellular tissue than in a syncytial mass of protoplasm. This may perhaps be correlated with the obvious fact that synthesized substances are more easily localized in cellular tissues. It might perhaps be possible to devise experiments to discover something about the advantages an organism obtains by keeping its protoplasm in amounts small enough for each to be related to a single nucleus.

The reason why even a somatically syncytial organism nearly always has unicellular gametes is of quite a different nature. Why should not a higher animal, for instance, reproduce by syncytial gametes, like those of *Rhizopus*? Let us suppose that the nuclei of the syncytial gametes of such an animal were the immediate products of meiosis. They would necessarily differ among themselves in their gene-complexes. When karyogamy had occurred, a wide assortment of gene-complexes would be present in the embryo. Let us suppose, for example, that one of these complexes was such as to be potentially favourable to the survival of the organism, if present in nerve-cells. It will at once be evident that the nuclei derived from the zygote nucleus carrying that particular complex might be absent from the nervous system and present only in other tissues, in which it could not exhibit its beneficial effects. There would only be two ways of overcoming this barrier to the action of natural selection and therefore to evolution. One possibility would be to form a large number of uninucleate spores, each capable of developing into a whole organism carrying the same gene-complex in every nucleus of the somatic tissues. (This is exactly what some of the Zygomycetes do—and without wasting much time on vegetative growth at this stage.) A much simpler and quicker way would be to reproduce sexually by uninucleate gametes.

Natural selection can only act effectively on an organism that has the same gene-complex in the nuclei of all its somatic tissues; and that can only be achieved by periodical reversion to the unicellular state.

kindly allowed copies to be made of illustrations in books and journals in their possession. Mr. Michael Lyster has made most of the photographs used in this paper. Several of them are clearer than the originals, from increase of contrast, but none has been retouched.

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