VI.

ON THE NUMBER OF POLAR BODIES AND THEIR SIGNIFICANCE IN HEREDITY.

1887.

ON THE NUMBER OF POLAR BODIES AND THEIR SIGNIFICANCE IN HEREDITY.

PREFACE.

THE following paper stands in close relation to a series of short essays which I have published from time to time since the year 1881. The first of these treated of 'The Duration of Life,' and the last of 'The Significance of Sexual Reproduction.' The present essay is most intimately connected with that upon 'The Continuity of the Germ-plasm,' and has, in fact, grown out of the explanation of the meaning of polar bodies in the animal egg, brought forward in that essay. The explanation rested upon a trustworthy and solid foundation, as I am now able to maintain with even greater confidence than at that time. It rested upon the idea that in the egg-cell, a cell with a high degree of histological differentiation, two different kinds of nuclear substance exert their influence, one after the other. But continued investigation has shown me that the explanation built upon this idea is only correct in part, and that it does not exhaust the full meaning of the formation of polar bodies. In the present essay I hope to complete the explanation by the addition of essential elements, and I trust that, at the same time, I shall succeed in throwing new light upon the mysterious problems of sexual reproduction and parthenogenesis.

It is obvious that this essay can only contain an attempt at an explanation, an hypothesis, and not a solution which is above criticism, like the results of mathematical calculation. But no biological theory of the present day can escape a similar fate, for the mathematical key which opens the door leading to the secrets of life has not yet been found, and a considerable period of time must elapse before its discovery. But although I can only offer an hypothesis, I hope to be able to show that it has not been rashly adopted, but that it has grown in a natural manner from the secure foundation of ascertained facts.

Nothing impresses the stamp of truth upon an hypothesis more than the fact that its light renders intelligible not only those facts for the explanation of which it has been framed, but also other and more distantly related groups of phenomena. This seems to me to be the case with my hypothesis, since the interpretation of polar bodies and the ideas derived from it unite from very different points of view, the facts of reproduction, heredity and even the transformation of species, into a comprehensive system, which although by no means complete, is nevertheless harmonious, and therefore satisfactory.

Only the most essential elements of the new facts which form the foundation of the views developed in this essay will be briefly mentioned. My object is to show all the theoretical bearings of these new facts, not to describe them in technical detail. Such a description accompanied by the necessary figures will shortly be given in another place[1].

A. W.

FREIBURG I. BR., *May* 30, 1887.

[1] See Berichten der Naturforschenden Gesellschaft zu Freiburg i. B., Band III. (1887) Heft 1, 'Ueber die Bildung der Richtungskörper bei thierischen Eiern,' by August Weismann and C. Ischikawa.

ON THE NUMBER OF POLAR BODIES, &c.

CONTENTS.

VI.

ON THE NUMBER OF POLAR BODIES AND THEIR SIGNIFICANCE IN HEREDITY.

I. Parthenogenetic and Sexual Egg.

Hitherto no value has been attached to the question whether an animal egg produces one or two polar bodies. Several observers have found two such bodies in many different groups of animals, both high and low in the scale of organization. In certain species only one has been observed, in others again three, four, or five (e. g. Bischoff, in the rabbit). Many observers did not even record the number of polar bodies found by them, and simply spoke of 'polar bodies.' As long as their formation was looked upon as a process of secondary physiological importance—as an 'excretion,' or a 'process of purification,' or even as the 'excreta' (!) of the egg, as a 'rejuvenescence of the nucleus,' or of mere historical interest as a reminiscence of ancestral processes, without any present physiological meaning—so long was it unnecessary to attach any importance to the number of these bodies, or to pay special attention to them. Of all the above-mentioned views, the one which explained polar bodies as a mere reminiscence of ancestral processes seemed to be especially well founded. Ten years ago we were far from being able to prove that polar bodies occurred in all animal eggs, and even in 1880, Balfour said in his excellent 'Comparative Embryology,' 'It is very possible, not to say probable, that such changes [the formation of polar bodies] are universal in the animal kingdom, but the present state of our knowledge does not justify us in saying so[1].'

Even at the present day we are not, strictly speaking, justified in making this assertion, for polar bodies have not yet been proved to occur in certain groups of animals, such as reptiles and birds; but they have been detected in the great majority of the large groups of the animal kingdom, and wherever they have been looked for

[1] Vol. I. p. 60.

with the aid of our modern highly efficient appliances, they have been found [1].

A *deeper insight into the process* of fertilization has above all led to a closer study of antecedent phenomena.

O. Hertwig [2] and Fol [3] showed that the formation of polar bodies was connected with a division of the nuclear substance of the egg. Hertwig and Bütschli [4] then proved that the body expelled from the egg possessed the nature of a cell, and thus led the way to the view that the formation of polar bodies is a process of cell-division, although a very unequal one. Even then there was no reason for attaching any special importance to the number of these bodies; nor should we have such a reason if we agreed with Minot [5], Balfour [6], and van Beneden in ascribing a high physiological significance to this process, and assumed that the expelled polar body is the male part of the previously hermaphrodite egg-cell. We should not know in what proportion the quantities of the 'male' and 'female' parts were present, and it would therefore be impossible to decide, *a priori*, whether the 'male' part had to be removed from the body of the egg-cell in one, two, or more portions.

Even after the view that the nuclear substance is the essential element in fertilization had gained ground—a view chiefly due to Strasburger's investigations on the process of fertilization in Phanerogams—and after Hertwig's opinion had been confirmed, that the process of fertilization is essentially the conjugation of nuclei, even then there appeared to be no reason why the *number* of divisions undergone by the nucleus of the mature egg should be looked upon as an essential feature.

[1] The most recent example of this kind is afforded by the excellent work of O. Schultze, 'Ueber die Reifung und Befruchtung des Amphibieneies,' Zeitschr. f. wiss. Zool., Bd. XLV. 1887. Schultze has proved that two polar bodies are expelled from the egg of the Axolotl and of the frog, although all previous observers, including O. Hertwig, had been unable to find them. Thus the latter authority states as the result of an investigation specially directed towards this point, that the nucleus is transformed in a peculiar manner ('Befruchtung des thierischen Eies,' III. p. 81).

[2] O. Hertwig, 'Beiträge zur Kenntniss der Bildung, Befruchtung, und Theilung des thierischen Eies,' Morpholog. Jahrbuch, I, II, and III. 1875–77.

[3] H. Fol, 'Recherches sur la fécondation et le commencement de l'hénogénie chez divers animaux.' Genève, Bâle, Lyon, 1879.

[4] Bütschli, 'Entwicklungsgeschichtliche Beiträge,' Zeitschr. f. wiss. Zool. Bd. XXIX. p. 237. 1877.

[5] C. S. Minot, 'Account, etc.' Proceedings Boston Soc. Nat. Hist., vol. xix. p. 165. 1877.

[6] F. M. Balfour, 'Comparative Embryology.'

This was the state of the subject at the time when I first made an attempt to ascertain the meaning of the formation of polar bodies. I based my views upon the idea, which was just then gaining ground, that Nägeli's idioplasm was to be sought for in the nucleus, and that the nucleoplasm must therefore contain the substance which determines the form and functions of the cell. Hence it followed that the germ-plasm—the substance which determines the course of embryonic development—must be identified with the nucleoplasm of the egg-cell. The conception of germ-plasm was brought forward by me before the appearance of Nägeli's work[1] which is so rich in fertile ideas; and germ-plasm does not exactly coincide with Nägeli's idioplasm[2]. Germ-plasm is only a certain kind of idioplasm—viz. that contained in the germ-cell—and it is the most important of all idioplasms, because all the other kinds are merely the results of the various ontogenetic stages into which it developes. I attempted to show that the molecular structure in these ontogenetic stages into which the germ-plasm developes would become more and more unlike that of the original structure of this substance, until it finally attains a highly specialized character at the end of embryonic development, corresponding to the production of specialized histological elements. It did not seem to me to be conceivable that the specialized idioplasm contained in the nuclei of the tissue cells could re-transform itself into the initial stage of the whole developmental series—that it could give up its specialized character and re-assume the generalized character of germ-substance. I will not repeat the reasons which induced me to adopt this opinion; they still seem to me to be conclusive. But let the above-mentioned theory be once accepted, and there follows from it another interesting conclusion concerning the germ-cell, or at least concerning those germ-cells which, like most animal eggs, possess a specific histological character. For obviously, such a character presupposes the existence of an idioplasm with a considerable degree of histological specialization, which must be contained in the nucleus of the egg-cell. We know, on the other hand, that when its growth is complete, after the formation of yolk and membranes, the egg contains

[1] Nägeli, 'Mechanisch-physiologische Theorie der Abstammungslehre,' München und Leipzig, 1884.

[2] See the second and fourth Essays in the present volume.

germ-plasm, for it is capable of developing into an embryo. We have therefore, as it were, two natures in a single cell, which become manifest one after the other, and which, according to our fundamental conception, can only be explained by the presence of two different idioplasms, which control the egg-cell one after the other, and determine its processes of development. At first a nucleoplasm leading to histological specialization directs the development of the egg and stamps upon it a specific histological character; and then germ-plasm takes its place, and compels the egg to undergo development into an embryo. If then the histogenetic or ovogenetic nucleoplasm of the egg-cell can be derived from the germ-plasm, but cannot be re-transformed into it (for the specialized can be derived from the generalized, but not the generalized from the specialized), we are driven to the conclusion that the germ-plasm, which is already present in the youngest egg-cell, first of all originates a specific histogenetic or ovogenetic nucleoplasm which controls the egg-cell up to the point at which it becomes mature; that its place is then taken by the rest of the unchanged nucleoplasm (germ-plasm), which has in the meantime increased by growth; and that the former is removed from the egg in the form of polar bodies—a removal which has been rendered possible by the occurrence of nuclear division. Hence the formation of polar bodies signified, in my opinion, the removal of the ovogenetic part of the nucleus from the mature egg-cell. Such removal was absolutely necessary, if it is impossible that the ovogenetic nucleoplasm can be re-transformed into germ-plasm. Hence the former substance cannot be made use of after the maturation of the egg, and it must even be opposed to the commencement of embryonic development, for it is impossible that the egg can be controlled by two forces of different kinds in the same manner as it would have been by one of them alone. I therefore concluded that the influence of the ovogenetic idioplasm must be removed before embryonic development can take place. In this way it seemed to me that not only the ordinary cases of ovogenetic and embryonic development became more easily intelligible, but also the rarer cases in which one and the same species produces two kinds of eggs—'summer and winter eggs.' Such eggs not only differ in size but also in the structure of yolk and membranes, although identical animals are developed from each of them. This result presupposes that the nucleus in both eggs contains identical germ-plasm, while the formation of different yolks and membranes requires the supposition that their nucleoplasm is different, inasmuch as the two eggs differ greatly in histological character.

The fact that equal quantities are separated during nuclear division, led me to conclude further that the expulsion of ovogenetic nucleoplasm can only take place when the germ-plasm in the nucleus of the egg-cell has increased by growth up to a point at which it can successfully oppose the ovogenetic nuclear substance. But we do not know the proportion which must obtain between the relative quantities of two different nuclear substances in order that nuclear division may be induced; and thus, by this hypothesis at least, we could not conclude with certainty as to the necessity for a single or a double division of the egg. It did not seem to be altogether inconceivable that the ovogenetic nucleoplasm might be larger in amount than the germ-plasm, and that it could only be completely removed by means of two successive nuclear divisions. I admit that this supposition caused me some uneasiness; but since nothing was known which could have enabled us to penetrate more deeply into the problem, I was satisfied, for the time being, in having found any explanation of the physiological value of polar bodies; leaving the future to decide not only whether such explanation were valid, but also whether it were exhaustive. The explanation seems to have found but little favour with some of our highest authorities. Hensen[1] does not consider that my reasons for the distinction between germ-plasm and histogenetic nucleoplasm are conclusive, and it may be conceded that this objection was perhaps, at that time, well founded. O. Hertwig does not mention my hypothesis at all in his work on embryology[2], although he states in the preface: 'Among current problems I have chiefly taken into consideration the views which seem to me to be most completely justified, but I have not left unmentioned the views which I cannot accept.' Minot's hypothesis is discussed by Hertwig, but Bütschli's[3] is preferred by him, although these two

[1] Hensen, 'Die Grundlagen der Vererbung,' Zeitschr. f. wiss. Landwirthschaft. Berlin, 1885, p. 749.

[2] O. Hertwig, 'Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere.' Jena, 1886.

[3] Bütschli, 'Gedanken über die morphologische Bedeutung der sog. Richtungskörperchen,' Biol. Centralblatt, Bd. VI. p. 5. 1884.

hypotheses are not strictly opposed to each other; for the former is a purely physiological, the latter a purely morphological explanation. I desire to lay especial stress upon the fact that my hypothesis is simply a logical consequence from the conclusion that the nuclear substance determines the nature of a cell. How this takes place is quite another question, which need not be discussed here. If it is only certain that the nature of a cell is thus determined, it follows that a cell with a certain degree of histological specialization must contain a nucleoplasm corresponding to the specialization. But the mature egg also contains germ-plasm, and there are only two possibilities by which these facts can be explained: either the ovogenetic nucleoplasm is capable of re-transformation into germ-plasm, or it is incapable of such re-transformation. Now, quite apart from the arguments which might be advanced in favour of one of these two possibilities, the fact that a body is undoubtedly expelled from the mature egg seems to me of importance, while it is of even greater importance that this body contains nucleoplasm from the germ-cell.

It may be thought that the process, as supposed by me, is without analogy, but such a conclusion is wrong, for during every embryonic development there are numerous cell-divisions in which unequal nucleoplasms are separated from one another, and in all these cases we cannot imagine any way in which the process can take place, except by supposing that the two kinds of nucleoplasm were previously united in the mother-cell, although their differentiation probably took place only a short time before cell-division. Perhaps the new facts which will be mentioned presently, and the views derived from them, will make my hypothesis upon the histogenetic nucleoplasm of the germ-cells appear in a more favourable light to the authorities above-named.

My hypothesis has at all events the one merit that it has led me to fruitful investigations.

If the formation of polar bodies really means the removal of ovogenetic nucleoplasm from the mature egg, they must also be found in parthenogenetic eggs; inasmuch as the latter possess a specific histological structure equal to that found in eggs requiring fertilization. If, therefore, it were possible to observe the formation of polar bodies in eggs which develope parthenogenetically, such an observation would not form a proof of the validity of my interpretation; but it would be a fact which harmonized with it, and negatived a suggestion which, if confirmed, would have been fatal to the hypothesis. Minot, Balfour, and van Beneden, from the point of view afforded by their theories, were compelled to suppose that polar bodies are wanting in parthenogenetic eggs; and the facts which were known at that time favoured such an opinion, for in spite of many attempts, no one had ever succeeded in proving the formation of these bodies by parthenogenetic eggs.

During the summer of 1885 I first succeeded in ascertaining that a single polar body is expelled from the parthenogenetic summer-egg of one of the *Daphnidae,—Polyphemus oculus*[1]. Thus my interpretation of the process in question received support, while it seemed to me that Minot's interpretation of polar bodies had been refuted; for if these bodies are formed in the parthenogenetic eggs of a single species, just as in eggs which require fertilization, it follows that the expulsion of polar bodies cannot signify the removal of the male element from the egg.

The desire to throw light upon the significance of polar bodies has been the only cause of my investigation. At the same time I hoped by this means to gain further knowledge as to the nature of parthenogenesis.

In the third part of the essay on 'The Continuity of the Germ-plasm' (see p. 225) I attempted to make clear the nature of parthenogenesis, and I arrived at the conclusion that the difference between an egg which is capable of developing without fertilization, and another which requires fertilization, must lie in the quantity of nucleoplasm present in the egg. I supposed that the nucleus of the mature parthenogenetic egg contained nearly twice as much germ-plasm as that contained in the sexual egg, just before the occurrence of fertilization; or, more correctly, I believed that the quantity of nucleoplasm which remains in the egg, after the expulsion of the polar bodies, is the same in both eggs, but that the parthenogenetic egg possesses the power of doubling this quantity by growth, and thus produces from within itself the same quantity of germ-plasm as that contained in the sexual egg after the addition of the sperm-nucleus in fertilization.

This was only an hypothesis, and the considerations which had led

[1] This observation was first published as a note at the end of the fourth Essay in the present volume. See p. 249.

to it depended, as far as they went into details, upon assumptions; but the fundamental view that the *quantity* of the nucleus decides whether embryonic development takes place with or without fertilization seemed to me, even at that time, to be correct, and to be a conclusion required by the facts of the case. Indeed, I thought it not unlikely that its validity might be proved by direct means: I pointed out that a comparison of the quantities of the nuclei in parthenogenetic and sexual eggs, if possible in the same species, would enable us to decide the question (*l. c.*, p. 234).

I had thus set myself the task of making this comparison. The result of this investigation was to show that, as already mentioned, polar bodies are formed in parthenogenetic eggs. But even the first species successfully investigated revealed a further fact, which, if proved to be wide-spread and characteristic of all parthenogenetic eggs, was certain to be of extreme importance:—the maturation of the parthenogenetic egg is accompanied by the expulsion of *one* polar body, or, as we might express it in another way, the substance of the female pronucleus is only *once* divided, and not *twice*, as in the sexual eggs of so many other animals. If this difference between parthenogenetic and sexual eggs was shown to be general, then the foundations of my hypothesis would indeed have been proved to be sound. The quantity of nuclear substance decides whether the egg is capable of undergoing embryonic development. This quantity is twice as large in the parthenogenetic as in the sexual egg. I had, however, been mistaken in a matter of detail; for the difference in the quantities of nuclear substance is not produced by the expulsion of two polar bodies, and the reduction of the nuclear substance to a quarter of its original amount, in both eggs, while the parthenogenetic egg then doubles its nuclear substance by growth; but the difference is produced because the reduction of nuclear substance originally present is less in one case than it is in the other. In the parthenogenetic egg the nuclear substance is only reduced to one-half by a single division; in the sexual egg it is reduced to a quarter by two successive divisions. It is an obvious conclusion from this fact, if proved to be wide-spread, that the significance of the first polar body must be different from that of the second. Only one polar body can signify the removal of ovogenetic nucleoplasm from the mature egg, and the second is obviously a reduction of the germ-plasm itself to half of its original amount. This very point seemed to me to be of great importance, because, as I had foreseen long ago, and as will be shown later on, the theory of heredity forces us to suppose that every fertilization must be preceded by a reduction of the ancestral idioplasms present in the nucleus of the parent germ-cell, to one-half of their former number.

But before the full bearing of the phenomena could be considered, it was necessary to ascertain how far they were of general occurrence. There were two ways in which this might be achieved, and in which it was possible to prove that parthenogenetic eggs expel only one polar body, while sexual eggs expel two. We might attempt to observe the phenomena of maturation in both kinds of eggs in a species which reproduces itself by the parthenogenetic as well as the sexual method. This would be the simplest way in which the question could be decided, if it were possible to make such observations on a sufficient number of species. But the other method was also open, a method which would have been the only one, if we did not know of any animals with two kinds of reproduction. We might attempt to investigate the phenomena of maturation in a large number of parthenogenetic eggs, if possible from different groups of animals, and we might compare the results with the facts which are already certain concerning the expulsion of polar bodies from the sexual eggs of so many species.

I have followed both methods, and by means of the second I have arrived already, indeed some time ago, at the certain conclusion that the above-mentioned difference is really general and without exception. The first polar body only is formed in all the parthenogenetic eggs which I investigated, with the valuable assistance of my pupil, Mr. Ischikawa of Tokio. On the other hand, an extensive examination of the literature of the subject convinced me that there is not a single undoubted instance of the expulsion of only one polar body from eggs which require fertilization, and that there are very numerous cases known from almost all groups of the animal kingdom in which it is perfectly certain that two polar bodies are formed, one after the other. A number of the older observations cannot be relied upon, for the presence of two polar bodies is mentioned without any explanation as to whether they are expelled from the egg one after the other, or whether they have merely resulted from the division of a single body after its expulsion. In parthenogenetic eggs two polar bodies are also formed in most cases, but they arise from the subsequent division of the single body which separates from the egg. But such subsequent division is only of secondary importance as far as the egg itself is concerned, and is also unimportant in the interpretation of the process. The essential nature of the process is to be found in the fact that the nucleus of the egg-cell only divides once when parthenogenesis occurs, but twice when fertilization is necessary, and it is of no importance whether the expelled part of the nucleus of the cell-body atrophies at once, or after it has undergone division. We have, therefore, to distinguish between primary and secondary polar bodies. If this distinction is recognized, and if we leave out of consideration all doubtful cases mentioned in literature, such a large number of well-established observations remain, that the existence of two primary polar bodies in sexual eggs, and neither a smaller nor a larger number, may be considered as proved.

Hence follows a conclusion which I believe to be very significant, —the difference between parthenogenetic and sexual eggs lies in the fact that in the former only one primary polar body is expelled, while two are expelled from the latter. When, in July, 1886, I published a short note [1] on part of the observations made upon parthenogenetic eggs, I confined myself to facts, and did not mention this conclusion. I took this course simply because I did not wish to bring it forward until I had made sufficient observations in the first of the two ways described above. I had hoped to be able to offer all the proofs that can be obtained before undertaking to publish the far-reaching consequences which would result from the above-mentioned conclusion. Unfortunately the material with which I had hoped to quickly settle the matter, proved less favourable than I had expected. Many hundred sections through freshly laid winter-eggs of *Bythotrephes longimanus* were made in vain; they did not yield the wished for evidence, and although continued investigation of other material has led to better results, the proofs are not yet entirely complete.

I should not therefore even now have brought forward the above-mentioned conclusion, if another observer had not alluded to this

[1] Weismann, 'Richtungskörper bei parthenogenetischen Eieren,' Zool. Anzeiger, 1886, p. 570.

idea, referring to my observations and also to a new discovery of his own. In a recent number of the 'Biologische Centralblatt,' Blochmann[1] gives an account of his continued observations upon the formation of polar bodies. It is well known that this careful observer had previously shown that polar bodies do occur in the eggs of insects, although they had not been found before. Blochmann proved that they are found in the representatives of three different orders, so that we may indeed 'confidently hope to find corresponding phenomena in other insects.' This discovery is most important, and it was naturally very welcome to me, as I had for a long time ascribed a high physiological importance to the process of the formation of polar bodies, and it would not be in accordance with such a view if the process was entirely wanting from whole classes of animals. To fill up this gap in our knowledge, and to give the required support to my theoretical views, I had proposed to one of my pupils, Dr. Stuhlmann[2], that he should work out the maturation of the eggs of insects; and it is a curious ill-luck that he, like many other observers, did not succeed in observing the expected expulsion of polar bodies, in spite of the great trouble he had taken. It may be that the species selected for investigation were unfavourable: at all events, we cannot now doubt that a division of the egg-nucleus is quite universal among insects, for Blochmann, in his latest contribution to the subject, proves that the *Aphidae* also form polar bodies. He examined the winter-eggs of *Aphis aceris*, and ascertained that they form two polar bodies, one after the other. Even in the viviparous *Aphidae*, thin sections revealed the presence of a polar body, though Blochmann could not trace all the stages of its development. It appears that the polar body is here preserved for an exceptional period, and its presence can still be proved when the blastoderm has been formed, and sometimes when development is even further advanced. Skilled observers of recent times, such as Will and Witlaczil, have not been able to find a polar body in the parthenogenetic eggs of the *Aphidae*, and

[1] Blochmann, 'Ueber die Richtungskörper bei den Insekteneiern,' Biolog. Centralblatt., April 15, 1887.

[2] F. Stuhlmann, 'Die Reifung des Arthropodeneies nach Beobachtungen an Insekten, Spinnen, Myriapoden und Peripatus,' Berichte der naturforschenden Gesellschaft zu Freiburg i. Br., Bd. I. p. 101.

Blochmann's proof of its existence seems to me to be of especial value, because the eggs of *Aphidae* are in many respects so unusually reduced; for instance, the primary yolk is absent and the egg-membrane is completely deficient, so that we might have expected that if polar bodies are ever absent, they would be wanting in these animals—that is, if they were of no importance, or at any rate of only secondary importance.

Hence the presence of polar bodies in *Aphidae* is a fresh confirmation of their great physiological importance. As bearing upon the main question dealt with in this essay, Blochmann's observations have an especial interest, because only one polar body was found in the parthenogenetic eggs of *Aphis*, while the sexual eggs normally produce two. The author rightly states that this result is in striking accordance with my results obtained from the summer-eggs of different *Daphnidae*, and he adds the remark,—'It would be of great interest to know whether these facts are due to the operation of some general law.' To this remark I can now reply that there is indeed such a law: not only in the parthenogenetic eggs of *Daphnidae*, but also, as I have since found, in those of the Ostracoda and Rotifera [1], only one primary polar body is formed, while two are formed in all eggs destined for fertilization.

Before proceeding to the conclusions which follow from this fact, I will at once remove a difficulty which is apparently presented by the eggs which may develope with or without fertilization. I refer to the well-known case of the eggs of bees. It might be objected to my theory that the same egg cannot be prepared for development in more than one out of the two possible ways; it might be argued that the egg either possesses the power of entering upon two successive nuclear divisions during maturation,

[1] In the summer-eggs of Rotifera I have, together with Mr. Ischikawa, observed one polar body, and we were able to establish for certain that a second is not formed. The nuclear spindle had already been observed by Tessin, and Billet had noticed polar bodies in *Philodina*, but without attaching any importance to their number. These latter observations were not conclusive proofs of the formation of polar bodies in parthenogenetic eggs, so long as it was not known whether the summer-eggs of Rotifera may develope parthenogenetically, or whether they can only develope in this way. Knowing now that parthenogenetic eggs expel only one polar body, we may perhaps be permitted to draw the conclusion that the summer-egg of a Rotifer (*Lacinularia*) which expelled only one polar body must have been a parthenogenetic egg. But I may add that we have also succeeded in directly proving the occurrence of parthenogenesis in Rotifera, as will be described in detail in another paper.

and in this case requires fertilization; or the egg may be of such a nature that it can only enter upon one such division and can therefore form only one polar body, and in that case it is capable of parthenogenetic development. Now there is no doubt, as I pointed out in my paper on the nature of parthenogenesis[1], that in the bee the very same egg may develope parthenogenetically, which under other circumstances would have been fertilized. Bessel's[2] experiments, in which young queens were rendered incapable of flight, and were thus prevented from fertilization, have shown that all the eggs laid by such females develope into drones (males) which are well known to result from parthenogenetic development. On the other hand, bee-keepers have long known that young queens which are fertilized in a normal manner continue for a long time to lay eggs which develope into females, that is to say, which have been fertilized. Hence the same eggs, viz. those which are lowest in the oviducts and are therefore laid first, develope parthenogenetically in the mutilated female, but are fertilized in the normal female. The question therefore arises as to the way in which the eggs become capable of adapting themselves to the expulsion of two polar bodies when they are to be fertilized, and of one only when fertilization does not take place.

But perhaps the solution of this problem is not so difficult as it appears to be. If we may assume that in eggs which are capable of two kinds of development the second polar body is not expelled until the entrance of a spermatozoon has taken place, the explanation of the possibility of parthenogenetic development when fertilization does not occur would be forthcoming. Now we know, from the investigations of O. Hertwig and Fol, that in the eggs of *Echinus* the two polar bodies are even formed in the ovary, and are therefore quite independent of fertilization, but in this and other similar cases a parthenogenetic development of the egg never takes place. There are, however, observations upon other animals which point to the fact that the first only and not the second polar body may be formed before the spermatozoon penetrates into the egg. It can be easily understood why it is that entirely conclusive observations are wanting, for hitherto there has been no reason for any accurate distinction

[1] See Essay IV, Part III. p. 225.

[2] E. Bessels, 'Die Landois'sche Theorie, widerlegt durch das Experiment.' Zeitschr. f. wiss. Zool. Bd. XVIII. p. 124. 1868.

between the first and the second polar body. Butin many eggs it appears certain that the second polar body is not expelled until the spermatozoon has penetrated. O. Schultze, the latest observer of the egg of the frog, in fact saw the first polar body alone extruded from the unfertilized egg: a second nuclear spindle was indeed formed, but the second polar body was not expelled until after fertilization had taken place. A very obvious theory therefore suggests itself:—that while the formation of the second polar body is purely a phenomenon of maturation in most animal eggs, and is independent of fertilization,—in the eggs of a number of other animals, on the other hand, and especially among Arthropods, the formation of the second nuclear spindle is the result of a stimulus due to the entrance of a spermatozoon. If this suggestion be confirmed, we should be able to understand why parthenogenesis occurs in certain classes of animals wherever the external conditions of life render its appearance advantageous, and further, why in so many species of insects a sporadic parthenogenesis is observed, viz. the parthenogenetic development of single eggs (Lepidoptera). Slight individual differences in the facility with which the second nuclear spindle is formed independently of fertilization would in such cases decide whether an egg is or is not capable of parthenogenetic development. As soon, however, as the second nuclear spindle is formed, parthenogenesis becomes impossible. The nuclear spindle which gives rise to the second polar body, and that which initiates segmentation, are two entirely different things, and although they contain the same quantity, and the same kind of germ-plasm, a transformation of the one into the other is scarcely conceivable. This conclusion will be demonstrated in the following part of the essay.

II. The Significance of the Second Polar Body.

I have already discussed the physiological importance of the first polar body, or rather of the first division undergone by the nucleus of the egg, and I have explained it as the removal of ovogenetic nuclear substance which has become superfluous and indeed injurious after the maturation of the egg. I do not indeed know of any other meaning which can be ascribed to this process, now that we know of the occurrence of a first division of the nucleus in parthenogenetic as well as in sexual eggs. A part of the nucleus must thus be removed from both kinds of eggs, a part which was necessary to complete their growth, and which then became superfluous and at the same time injurious. In this respect the observations of Blochmann [1] upon the eggs of *Musca vomitoria* seem to me to be very interesting. Here the two successive divisions of the nuclear spindle arising from the egg-nucleus take place, but true polar bodies are not expelled, and the two nuclei corresponding to them (one of which divides once more) are placed on the surface of the egg, surrounded by an area free from yolk granules; and they break up at a later period. The essential point is obviously to eliminate from the egg-cell the influence of nucleoplasm which has been separated from the egg-nucleus as the first polar body; and this condition is satisfied whether the elimination is brought about by a process of true cell-division, as is the rule in the eggs of most animals, or by the division and removal of part of the egg-nucleus alone. The occurrence of the latter method of elimination certainly constitutes a still further proof of the physiological importance of the process, and this, taken together with the universal occurrence of polar bodies in all eggs—parthenogenetic and sexual—forces us to conclude that the process must possess a definite significance. No one of the various attempts which have been made to explain the significance of polar bodies generally is applicable to the *first* polar body except that which I have attempted.

But the case is different with the significance of the *second* nuclear division, or the *second* polar body. Here it might perhaps be possible to return to the view brought forward by Minot, Balfour, and van Beneden, and to consider the removal of this part of the nucleus as the expulsion of the male part of the previously hermaphrodite egg-cell. The second polar body is only expelled when the egg is to be fertilized, and at first sight it appears to be quite obvious that such a preparation of the egg for fertilization must depend upon its reduction to the female state. I believe however that this is not the case, and am of opinion that the process has an entirely different and much deeper meaning.

How can we gain any conception of this supposed hermaphroditism of the egg-cell, and its subsequent attainment of the

[1] l. c., p. 110.

female state? What are the essential characteristics of the male and female states? We know of female and male individuals, among both animals and plants: their differences consist essentially in the fact that they produce different kinds of reproductive cells; in part they are of a secondary nature, being adaptations of the organism to the functions of reproduction; they are intended to attract the other sex, or to ensure the meeting of the two kinds of reproductive cells, or to enable the fertilized egg to develope and sometimes to guide the development of the offspring until it has reached a certain period of growth. But all these differences, however great they may sometimes be, do not alter the essential nature of the organism. The blood corpuscles of man and woman are the same, and so are the cells of their nerves and muscles; and even the sexual cells, so different in size, appearance, and generally also in motile power, must contain the same fundamental substance, the same idioplasm. Otherwise the female germ-cell could not transmit the male characters of the ancestors of the female quite as readily as the female characters, nor could the male germ-cell transmit the female quite as readily as the male characters of the ancestors of the male. It is therefore clear that the nuclear substance itself is not sexually differentiated.

I have already previously pointed out that the above-mentioned facts of heredity contain the disproof of Minot's theory, inasmuch as the egg-cell transmits male as well as female characters. Strasburger [1] has also raised a similar objection. I consider this objection to be quite conclusive, for there does not seem to be any way in which the difficulty can be met by the supporters of the theory. The difficulty could indeed be evaded until we came to know that the essential part of the polar body is nuclear substance, and that the latter must be regarded as idioplasm,—as the substance which is the bearer of heredity. It might have been maintained that the male part, removed from the egg, consists only in a condition, perhaps comparable to positive or negative electricity; and that this condition is present in the substance of the polar body, so that the removal of the latter would merely signify a removal of the unknown condition. I do not mean to imply that any of those who have adopted Minot's theory have had any such vague ideas

[1] Strasburger, 'Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage einer Theorie der Zeugung.' Jena, 1884.

concerning this process, but even if any one were ready to adopt it, he would be unable to make any use of the idea. He would not be able to support the theory in this way, for we now know that nuclear substance is removed with the polar body, and this fact requires an explanation which cannot be afforded by the theory, if we are right in believing that the expelled nuclear substance is not merely the indifferent bearer of the unknown principle of the male condition, but hereditary substance. I therefore believe that Minot's, Balfour's, and van Beneden's hypothesis, although an ingenious attempt which was quite justified at the time when it originated, must be finally abandoned.

My opinion of the significance of the second polar body is shortly this,—a reduction of the germ-plasm is brought about by its formation, a reduction not only in quantity, but above all in the complexity of its constitution. By means of the second nuclear division the excessive accumulation of different kinds of hereditary tendencies or germ-plasms is prevented, which without it would be necessarily produced by fertilization. With the nucleus of the second polar body as many different kinds of idioplasm are removed from the egg as will be afterwards introduced by the sperm-nucleus; thus the second division of the egg-nucleus serves to keep constant the number of different kinds of idioplasm, of which the germ-plasm is composed during the course of generations.

In order to make this intelligible a short explanation is necessary.

From the splendid series of investigations on the process of fertilization, commenced by Auerbach and Bütschli, and continued by Hertwig, Fol, Strasburger, van Beneden, and many others, and from the theoretical considerations brought forward by Pflüger, Nägeli, and myself, at least one certain result follows, viz. that there is an hereditary substance, a material bearer of hereditary tendencies, and that this substance is contained in the nucleus of the germ-cell, and in that part of it which forms the nuclear thread, which at certain periods appears in the form of loops or rods. We may further maintain that fertilization consists in the fact that an equal number of loops from either parent are placed side by side, and that the segmentation nucleus is composed in this way. It is of no importance, as far as this question is concerned, whether the loops of the two parents coalesce sooner or later, or whether they remain separate. The only essential conclusion demanded by our hypothesis is that there should be complete or approximate equality between the quantities of hereditary substance derived from either parent. If then the germ-cells of the offspring contain the united germ-plasms of both parents, it follows that such cells can only contain half as much paternal germ-plasm as was contained in the germ-cells of the father, and half as much maternal germ-plasm as was contained in the germ-cells of the mother. This principle is affirmed in a well-known calculation made by breeders of animals, who only differ from us in their use of the term 'blood' instead of the term germ-plasm. Breeders say that half of the 'blood' of the offspring has been derived from the father and the other half from the mother. The grandchild similarly derives a quarter of its 'blood' from each of the four grandparents, and so on.

Let us imagine, for the sake of argument, that sexual reproduction had not been introduced into the animal kingdom, and that asexual reproduction had hitherto existed alone. In such a case, the germ-plasm of the first generation of a species which enters upon sexual reproduction must still be entirely homogeneous; the hereditary substance must, in each individual, consist of many minute units, each of which is exactly like the other, and each of which contains within itself the tendency to transmit, under certain circumstances, the whole of the characters of the parent to a new organism—the offspring. In each of the offspring of such a first generation, the germ-plasms of two parents will be united, and every germ-cell contained in the individuals of this second sexually produced generation will now contain two kinds of germ-plasm—one kind from the father, and the other from the mother. But if the total quantity of germ-plasm present in each cell is to be kept within the pre-determined limits, each of the two ancestral germ-plasms, as I may now call them, must be represented by only half as many units as were contained in the parent germ-cells.

In the third sexually produced generation, two new ancestral germ-plasms would be added by fertilization to the two already present, and the germ-cells of this generation would therefore contain four different ancestral germ-plasms, each of which would constitute a quarter of the total quantity. In each succeeding generation the number of the ancestral germ-plasms is doubled, while their quantities are reduced by one half. Thus in the fifth sexually produced generation, each of the sixteen ancestral germ-plasms will only constitute $\frac{1}{16}$ of the total quantity; in the sixth, each of the thirty-two ancestral germ-plasms, only $\frac{1}{32}$, and so on. The germ-plasm of the tenth generation would be composed of 1024 different ancestral germ-plasms, and that of the n^{th} of 2^n. By the tenth generation each single ancestral germ-plasm would only form $\frac{1}{1024}$ of the total quantity of germ-plasm contained in a single germ-cell. We know nothing whatever of the length of time over which this process of division of the ancestral germ-plasms may have endured, but even if it had continued to the utmost possible limit—so far indeed that each ancestral germ-plasm was only represented by a single unit—a time would at last come when any further division into halves would cease to be possible; for the very conception of a unit implies that it cannot be divided without the loss of its essential nature, which in this case constitutes it as the hereditary substance.

In the diagram represented in Fig. I. I have tried to render these conclusions intelligible. In generation I. each paternal and maternal germ-plasm is still entirely homogeneous, and does not contain any combination of different hereditary qualities, but the germ-plasm of the offspring is made up of equal parts of two kinds of germ-plasm. In the second generation this latter germ-plasm unites with another derived from other parents, which is similarly composed of two ancestral germ-plasms, and the resulting third generation now contains four different ancestral germ-plasms in its germ-cells, and so on. The diagram only indicates the fusion of ancestral germ-plasms as far as the offspring of the fourth generation, the germ-cells of which contain sixteen different ancestral germ-plasms. If we imagine the germ-plasm units to be so large that there is only room for sixteen of them in the nuclear thread, the limits of division would be reached in the fifth generation, and any further division into halves of the ancestral germ-plasms would be impossible.

Now however minute the units may be, there is not the least doubt that the limits of possible division have been long since reached by all existing species, for we may safely assume that no one of them has acquired the sexual method of reproduction within a small number of recent generations. All existing species must therefore now contain as many different kinds of ancestral germ-plasms as they are capable of containing; and the question arises,—How can sexual reproduction now proceed without a doubling of the quantity of germ-plasm in each germ-cell, with every new generation?

There is only one possible answer to such a question:—sexual reproduction can proceed by a reduction in the *number* of ancestral germ-plasms, a reduction which is repeated in every generation.

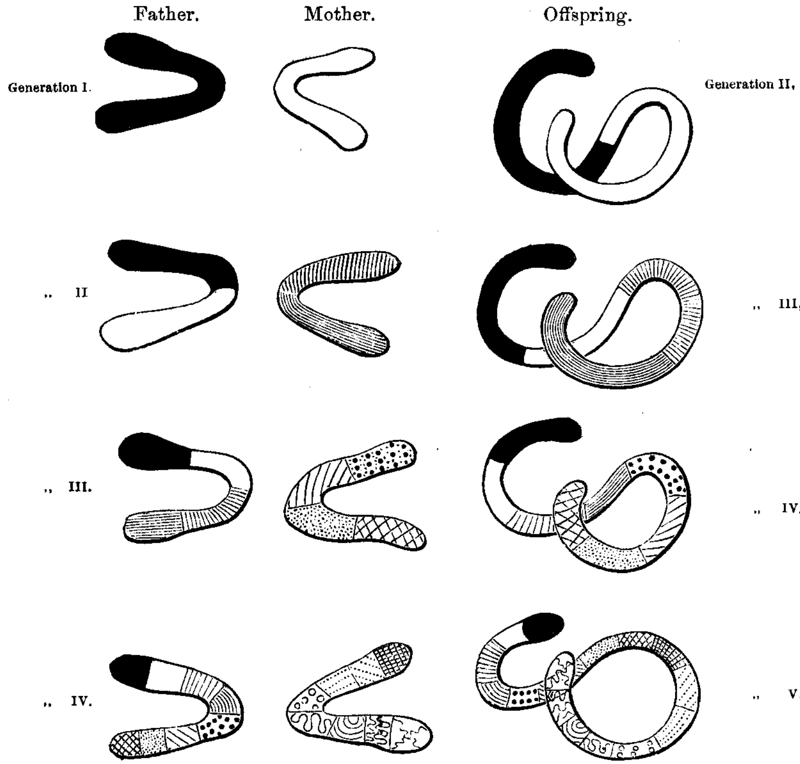


FIG. I.

This *must* be so: the only question is, how and when does the supposed reduction take place.

Inasmuch as the germ-plasm is seated, according to our theory, in the nucleus, the necessary reduction can only be produced by nuclear division; and quite apart from any observation which has been already made, we may safely assert that there *must* be a form of nuclear division in which the ancestral germ-plasms contained in the nucleus are distributed to the daughter-nuclei in such a way that each of them receives only half the number contained in the original nucleus. After Roux's[1] elaborate review of the whole subject, we need no longer doubt that the complex method of nuclear division, hitherto known as karyokinesis, must be considered not merely as a means for the division of the total quantity of nuclear substance, but also for producing a division of the quantity and quality of each of its single elements. In by far the greater number of instances the object of this division is obviously to effect an equal distribution of nuclear substance in the two daughter-nuclei, so that each of the different qualities contained in the mother-nucleus is transferred to the two daughter-nuclei. This interpretation of ordinary karyokinesis is less uncertain than perhaps at first sight it may appear to be. We cannot, it is true, directly see the ancestral germ-plasms, nor do we even know the parts of the nucleus which are to be looked upon as constituting ancestral germ-plasm; but if Flemming's original discovery of the longitudinal division of the loops lying in the equatorial plane of the nuclear spindle is to have any meaning at all, its object must be to divide and distribute the different kinds of the minutest elements of the nuclear thread as equally as possible. It has been ascertained that the two halves produced by the longitudinal splitting of each loop never pass into the same daughter-nucleus, but always in opposite directions. The essential point cannot therefore be the division of the nucleus into absolutely equal quantities, but it must be the distribution of the different qualities of the nuclear thread, without exception, in both daughter-nuclei. But these different qualities are what I have called the ancestral germ-plasms, i.e. the germ-plasms of the different ancestors, which must be contained in vast numbers, but in very minute quantities, in the nuclear thread. The supposition of a vast number is not only required by the phenomena of heredity but also results from the comparatively great length of the nuclear thread: furthermore it implies that each of them is present in very small quantity. The vast number together with the minute quantity of the ancestral germ-plasms permit us to conclude that they are, upon the whole, arranged in a linear manner in the thin thread-like loops: in fact the longitudinal

[1] Wilhelm Roux, 'Ueber die Bedeutung der Kerntheilungsfiguren.' Leipzig, 1884.

splitting of these loops appears to me to be almost a proof of the existence of such an arrangement, for without this supposition the process would cease to have any meaning.

This is the only kind of karyokinesis which has been observed until recently; but if the supposed nuclear division leading to a reduction in the number of ancestral germ-plasms has any real existence, there must be yet another kind of karyokinesis, in which the primary equatorial loops are not split longitudinally, but are separated without division into two groups, each of which forms one of the two daughter-nuclei. In such a case the required reduction in the number of ancestral germ-plasms would take place, for each daughter-nucleus would receive only half the number which was contained in the mother-nucleus.

Now there is more evidence for the existence of this second kind of karyokinesis than the fact that it is demanded by my theory; for I believe that it has been already observed, although it has not been interpreted in this sense.

It is very probable that this is true of van Beneden's[1] observation on the egg of *Ascaris megalocephala*: he found that the nuclear division which led to the formation of the polar body differs from the ordinary course of karyokinesis, in that the plane of division is at right angles to that usually assumed. Carnoy[2] has confirmed this observation in its main features, and he has made the further observation that out of the eight nuclear loops which are found at the equator of the spindle, four are removed with the first polar body, and that half of the remaining four are removed with the second polar body. The first of these two divisions would have to be looked upon as a reduction, if it is certain that each of the eight nuclear loops consists of different ancestral germ-plasms; but this assumption is impossible, although on the other hand it cannot be *directly* disproved: for we are not able to see the ancestral germ-plasms. But it must nevertheless be maintained that the removal of the first four loops does not imply a reduction in the number of ancestral germ-plasms in the nucleus; because, as I have already argued, two successive divisions of the number of ancestral germ-

[1] E. van Beneden, 'Recherches sur la maturation de l'œuf, la fécondation et la division cellulaire.' Gand et Leipzig, Paris, 1883.

[2] J. B. Carnoy, 'La Cytodiérèse de l'œuf, la vésicule germinative et les globules polaires de l'Ascaris megalocephala.' Louvain, Gand, Lierre, 1886.

plasms into halves is inconceivable; and because the first polar body is also present in parthenogenetic eggs in which such division into halves cannot take place. But the karyokinetic process can readily be looked upon as a removal of ovogenetic nucleoplasm, for we know from the observations of Flemming and Carnoy, that, under certain circumstances, subsequent divisions may occur, involving an increase in the number of nuclear loops to double their number. These subsequent divisions of course take place in the daughter-nuclei. This fact proves, as I think, that there are nuclei in which the same ancestral germ-plasm occurs in two different loops: but such loops, identical as regards the composition of their ancestral germ-plasms, may very well contain different ontogenetic stages of this substance. This will be the case in the instance alluded to, if four loops of the first nuclear spindle are to be looked upon as ovogenetic nucleoplasm, and the four others as germ-plasm. It is therefore unnecessary to regard the first division of the egg-nucleus as a 'reducing division': it may be looked upon as an 'equal division'[1] entirely analogous to the kind of division which, in my opinion, directs the development of the embryo. This conclusion would receive direct proof if it were possible to show that the eight loops of the first division have arisen by the longitudinal splitting of four *primary* loops: for a longitudinal splitting of the nuclear thread would be the means by which the different ontogenetic stages of the germ-plasm could be separated from one another, without leading to any reduction in the number of ancestral germ-plasms in the daughter-nuclei. Thus I have previously attempted to prove that the ontogenetic development of the egg must be connected with a progressive transformation of the nucleoplasm during successive nuclear divisions, and this transformation will very frequently (but not always) occur in such a way that the different qualities of the nucleoplasm are separated from one another by the nuclear division. The nucleoplasm of the daughter-nuclei will be identical if the two daughter-cells are to potentially contain corresponding parts of the embryo; as for instance the first two segmentation spheres of the egg of the frog, which according to Roux[2] correspond to the right and left halves of the future animal.

[1] See p. 364.

[2] Wilhelm Roux, 'Beiträge zur Entwicklungsmechanik des Embryo,' No. 3, Breslauer ärztliche Zeitschrift, 1885, p. 45.

But the nucleoplasm must be unequal if the products of division are to develope into different parts of the embryo. In both cases, however, karyokinesis is connected with a longitudinal splitting of the nuclear threads, and we may conclude from this fact (which is also confirmed by the phenomena of heredity) that all such nuclei, whether they have entered upon the same or different ontogenetic transformations of their nucleoplasm, are identical as regards the ancestral germ-plasm which they contain. During the whole process of segmentation and the entire development of the embryo, the total number of ancestral germ-plasms which were at first contained in the germ-plasm of the fertilized egg-cell must still be contained in each of the succeeding cells.

Thus no objection can be raised against the view that the four loops of the first polar body contain the ovogenetic nucleoplasm, that is to say, an idioplasm which contains the total number of ancestral germ-plasms, but at an advanced and highly specialized ontogenetic stage.

The formation of the second polar body may be rightly considered as a 'reducing division,' as a division leading to the expulsion of half the number of the different ancestral germ-plasms, in the form of two nuclear loops, for no reason can be alleged in support of the assumption that the four loops of the second nuclear spindle are made up of identical pairs. Furthermore the facts of heredity require the assumption that the greatest possible number of ancestral germ-plasms is accumulated in the germ-plasm of each germ-cell, and thus that the small number of loops not only means an increase in quantity but a multiplication in the number of different ancestral germ-plasms present in each of them. If this conclusion be correct, there can be no doubt that the second division of the egg-nucleus means a reduction in the above-mentioned sense.

But there are yet other observations which, if correct, must also be considered as 'reducing divisions.' I refer to all those cases in which the longitudinal splitting of the loops is either entirely wanting, or does not occur until after the loops have left the equator of the spindle and have moved towards the poles. In both instances the bearing upon the question would be the same, for only half the number of primary loops would reach each pole in either case. If therefore the primary loops are not made up of identical pairs, it follows that the two daughter-nuclei can only contain half the number of ancestral germ-plasms which were contained in the mother-nucleus. Whether the loops divide on their way to the poles or at the poles themselves, no difference will be brought about in the number of ancestral germ-plasms which they contain, for this number can neither increase nor diminish. The *quantity* of the different ancestral germ-plasms can alone be increased in this way. I am here referring to observations made by Carnoy[1] on the cells which form the spermatozoa in various Arthropods. It must be admitted, however, that these divisions cannot be regarded as 'reducing divisions,' if Flemming's[2] suggestion be confirmed, that in all these observations the fact has been overlooked that the equatorial loops are not primary but secondary, and that they have arisen from the longitudinal splitting of the nuclear thread during previous stages of nuclear division. But this point can only be decided by renewed investigation. Although many excellent results have been obtained in the subject of karyokinesis, there is still very much to be learnt before our knowledge is complete; and this is not to be wondered at when we remember the great difficulties in the way of observation which are chiefly raised by the minute size of the objects to be investigated. Flemming's most recent publications prove that we are still in the midst of investigation, and that highly interesting and important processes have hitherto escaped attention. A secure basis of facts is only very gradually obtained, and there are still many conflicting opinions upon the details of this process. I should therefore consider it to be entirely useless, from my point of view, to enter into a critical examination of everything known about all the details of karyokinesis. I am quite content to have shown how it may be imagined that the reduction required by my theory takes place during nuclear division; and at the same time to have pointed out that there are already observations which may be interpreted in this sense. But even if I am mistaken in this interpretation, the theoretical necessity for a reduction in the number of ancestral germ-plasms, a reduction repeated in every generation, seems to me to be so securely founded that the processes by which it is effected *must* take place, even if they are not supplied by the facts already ascertained. There must be two kinds of karyo-

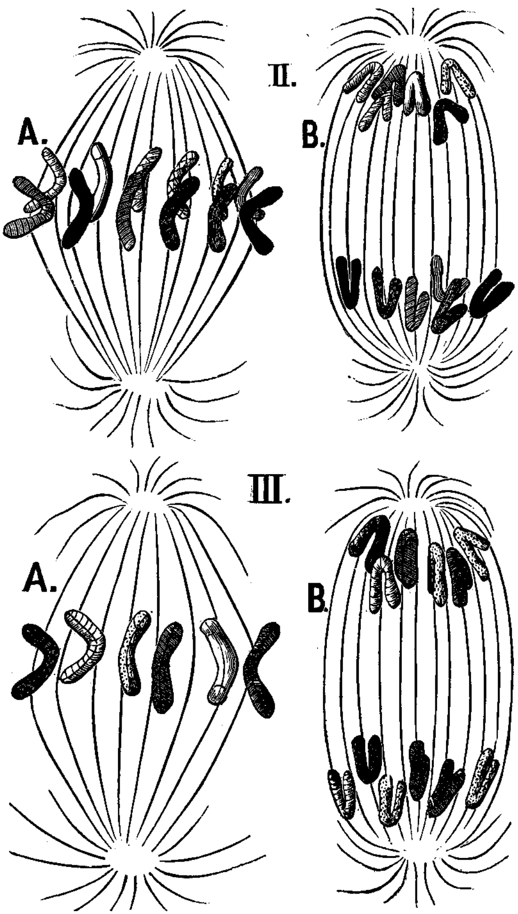
[1] Carnoy, 'La Cytodiérèse chez les Arthropodes.' Louvain, Gand, Lierre, 1885.

[2] Flemming, 'Neue Beiträge zur Kenntniss der Zelle.' Arch. f. mikr. Anat. Bd. XXIX, 1887.

kinesis according to the different physiological effect of the process. First, a karyokinesis by means of which all the ancestral germ-plasms are equally distributed in each of the two daughter-nuclei after having been divided into halves: secondly, a karyokinesis by means of which each daughter-nucleus receives only half the number of ancestral germ-plasms possessed by the mother-nucleus. The former may be called 'equal division,' the latter 'reducing division.' Of course these two processes, which differ so greatly in their effects, must also be characterized by morphological differences, but we cannot assume that the latter are necessarily visible. Just as, during the division of the first and second nuclear spindle in the egg of *Ascaris megalocephala*, karyokinesis takes, upon the whole, the same morphological course, although we must ascribe different physiological meanings to the two processes of division,—so it may be in other cases. The 'reducing division' must be always accompanied by a reduction of the loops to half their original number, or by a transverse division of the loops (if such division ever occurs); although reduction can only occur when the loops are not made up of identical pairs. And it will not always be easy to decide whether this is the case. On the other hand, the form of karyokinesis in which a longitudinal splitting of the loops takes place *before* they separate to form the daughter-nuclei must always, as far as I can see, be considered as an 'equal division.' In the accompanying figures II and III, diagrams are given illustrating these two forms of karyokinesis, but I do not mean to imply that it is impossible to imagine any other form in which they may occur.

In Figure II a nuclear spindle is seen at *A*, and at its equatorial zone there are twelve primary loops. The transverse cross-lines and other markings on the loops indicate that they are composed of different ancestral germ-plasms. The loops are shaded differently in order to render the diagram clear. At *B* six of the loops are seen to have moved to either pole, so that the figure is a representation of the 'reducing division.' Figure III is a diagrammatic representation of 'equal division.' The six loops at the equatorial zone of *A* are shown by different cross-lining and shading to be composed of different ancestral germ-plasms. The loops split longitudinally in a direction indicated by the longitudinal line upon each of them. In *B* the halves of the loops are seen to have moved to the opposite poles of the spindle, so that there are not only six loops at each pole, but also all the six combinations of ancestral germ-plasms.

Perhaps some may be inclined to look upon direct nuclear division as a 'reducing division,' but I believe that such a view



FIGS. II, III.

would be incorrect. It is only approximately true that the nuclear thread is divided into two halves of equal quantity by direct division, and exact equality would only happen as it were accidentally; so that we cannot speak of a perfectly equal distribution of the ancestral germ-plasm in the two daughter-nuclei. But the 'reducing division' must obviously effect an exactly regular and uniform distribution of the ancestral germ-plasms, although this does not imply that every ancestral germ-plasm of the mother-nucleus would be represented in each of the two daughter-nuclei. But if out of e.g. eight nuclear loops at the equatorial plane, four pass into one, and the other four into the other daughter-nucleus, each of the latter will contain an equal number of ancestral germ-plasms, although different ones. This is indeed part of the foundation of the theory, for the 'reducing division' must remove exactly half of the original number of ancestral germ-plasms, and precisely the same number must be replaced at a later period by the sperm-nucleus. This could hardly be achieved with sufficient precision by direct nuclear division.

I now come to inquire whether the expulsion of the second polar body is in reality, as I have already maintained, a reduction in the number of ancestral germ-plasms present in the nucleus of the egg. The view itself is sufficiently obvious, and it would supply an explanation of the meaning of the process which is still greatly wanted; but it will nevertheless be not entirely useless to consider other possible theories.

It would be quite conceivable to suppose that the youngest egg-cells, which multiply by division, may undergo one 'reducing division' in addition to the ordinary process. Of course this should occur once only, for if repeated, the number of ancestral idioplasms in the nucleus of the germ-cell would undergo a decrease greater than could be afterwards compensated by the increase due to fertilization. Thus the number of ancestral germ-plasms would continually decrease in the course of generations,—a process which would necessarily end with their complete reduction to a single kind, viz. to the paternal or the maternal germ-plasm. But the occurrence of such a result is disproved by the facts of heredity. Although such an early occurrence of the 'reducing division' would offer advantages in that nothing would be lost, for both daughter-nuclei would become eggs, instead of one of them being lost as a polar body, nevertheless I do not believe that it really occurs: weighty reasons can be alleged against it.

Above all, the facts of parthenogenesis are against it. If the number of ancestral germ-plasms received from the parents were reduced to half in the ovary of the young animal, how then could parthenogenetic development ever take place? It is true that we cannot at once assert the impossibility of an early 'reducing division' on this account, for as I have shown above, the power to develope parthenogenetically depends upon the quantity of germ-plasm contained in the mature egg; the necessary amount might be produced by growth, quite independently of the number of different kinds of ancestral germ-plasms which form its constituents. The size of a heap of grains may depend upon the number of grains, and not upon the number of different kinds of grains. But in another respect such a supposition would lead to an unthinkable conclusion. In the first place, the number of ancestral germ-plasms in the germ-cells would be diminished by one half in each new generation arising by the parthenogenetic method; thus after ten generations only $\frac{1}{1024}$ of the original number of ancestral germ-plasms would be present.

Now, it might be supposed that the 'reducing division' of the young egg-cells was lost at the time when the parthenogenetic mode of reproduction was assumed by a species; but this suggestion cannot hold, because there are certain species in which the same eggs can develope either sexually or parthenogenetically (e.g. the bee). It seems to me that such cases distinctly point to the fact that the reduction in the number of ancestral germ-plasms must take place immediately before the commencement of embryonic development, or, in other words, at the time of maturation of the egg. It is only decided at this time whether the egg of the bee is to develope into an embryo by the parthenogenetic or the sexual method; such decision being brought about, as was shown above, by the fact that only one polar body is expelled in the first case, while two are expelled in the second. But if we are obliged to assume that reproduction by means of fertilization, necessarily implies a reduction to one half of the number of ancestral germ-plasms inherited from the parents,—the further conclusion is obvious, that the second division of the egg-nucleus and the expulsion of the second polar body represent such a reduction, and that this second division of the egg-nucleus is unequal in the sense mentioned above, viz. one half of the ancestral germ-plasms remains in the egg-nucleus, the original number being subsequently restored by conjugation with a sperm-nucleus; while the other half is expelled in the polar body and perishes.

I may add that observations, so far as they have extended to such minute processes, do indeed prove that the number of loops is reduced to one half. It has been already mentioned that, according to Carnoy, such reduction occurs in *Ascaris megalocephala*, but the same author also describes the process of the formation of polar bodies in a large number of other *Nematodes*[1], and his descriptions show that the process occurs in such a way that the number of ancestral germ-plasms must be reduced by half. Sometimes half the number of primary loops pass into the nucleus of the polar body, while the other half remains in the egg. In other cases, as in *Ophiostomum mucronatum*, the primary nuclear rods divide transversely,—a process which must produce the same effect. It is true that these observations require confirmation, and since, with unfavourable objects, the difficulties of observation are extremely great, there may have been errors of detail; but I do not think that there is any reason for doubting the accuracy of the essential point. And this essential point is the fact that the number of primary loops is divided into half by the formation of the polar body.

But even if we could not admit that such a conclusion is securely founded, it cannot be doubted that the formation of the second polar body reduces to one half the quantity of the nucleus which would have become the segmentation-nucleus in the parthenogenetic development of the egg. This is a simple logical conclusion from the two following facts: first, parthenogenetic eggs expel only *one* polar body; secondly, there are eggs (such as those of the bee) in which it is absolutely certain that the same half of the nucleus—which is expelled as the second polar body in the egg requiring fertilization—remains in the egg when it is to develope parthenogenetically, and acts as half of the segmentation-nucleus. But this proves that the expelled half of the nucleus must consist of true germ-plasm, and thus a secure foundation is laid for the assumption that the formation of the nucleus of the second polar body must be considered as a 'reducing division.'

I was long ago convinced that sexual reproduction must be connected with a reduction in the number of ancestral germ-plasms to one half, and that such reduction was repeated in each generation. When, in 1885, I brought forward my theory of the conutinuity

[1] Carnoy, 'La Cytodiérèse de l'œuf; la vésicule germinative et les globules polaires chez quelques Nématodes.' Louvain, Gand, Lierre. 1886.

of the germ-plasm, I had long before that time considered whether the formation and expulsion of polar bodies must not be interpreted in this sense. But the two divisions of the egg-nucleus caused me to hesitate. The two divisions did not seem to admit of such an interpretation, for by it the quantity of the nucleus is not divided into halves, but into quarters. But a division of the number of ancestral germ-plasms into quarters would have caused, as was shown above, a continuous decrease, leading to their complete disappearance; and such a conclusion is contradicted by the facts of heredity. For this reason I was led at that time to oppose Strasburger's view that the expulsion of the polar bodies means a reduction of the quantity of nuclear substance by only half. My objection to such a view was valid when I said that the quantity of idioplasm contained in the egg-nucleus is not, as a matter of fact, reduced to one half, but to one quarter, inasmuch as two successive divisions take place. I may add that I had also considered whether the two successive divisions might not possess an entirely different meaning,—whether one of them led to the removal of ovogenetic nucleoplasm, while the other resulted in a reduction in the number of ancestral germ-plasms. But at that time there were no ascertained facts which supported the supposition of such a difference, and I did not wish to bring forward the idea, even as a suggestion, when there was no secure foundation for it. The morphological aspects of the formation of the first and second polar bodies are so extremely similar that such a supposition might have been considered as a mere effort of the imagination.

Hensen[1] also rejected the second part of the supposition that reduction must take place in the number of the hereditary elements of the egg, and that such reduction is caused by the expulsion of polar bodies, because he believed it to be incompatible with the fact, which had just been discovered, that polar bodies are formed by parthenogenetic eggs. He concludes with these words: 'If this striking fact be confirmed, the hypothesis which assumes that the egg must be divided into half before maturation, is refuted, and there only remains the rather vague explanation that a process of purification must precede the development of the embryo.'

[1] Hensen, 'Die Grundlagen der Vererbung nach dem gegenwärtigen Wissenskreis,' Zeitschr. f. wissenschaftl. Landwirthschaft, Berlin, 1885, p. 731.

Nevertheless Hensen is the only writer who has hitherto taken into consideration the idea that sexual reproduction causes a regularly occurring 'diminution in the hereditary elements of the egg.'

III. The Foregoing Considerations applied to the Male Germ-Cells.

If the result of the previous considerations be correct, and if the number of ancestral germ-plasms contained in the nucleus of the egg-cell destined for fertilization must be reduced by one half, there can be no doubt that a similar reduction must also take place, at some time and by some means, in the germ-plasms of the male germ-cells. This must be so if we are correct in maintaining that the young germ-cells of a new individual contain the same nuclear substance, the same germ-plasm, which was contained in the fertilized egg-cell from which the individual has been developed. The young germ-cells of the offspring must contain this substance if my theory of the continuity of the germ-plasm be well founded, for this theory supposes that, during the development of a fertilized egg, the whole quantity of germ-plasm does not pass through the various stages of ontogenetic development, but that a small part remains unchanged, and at a later period forms the germ-cells of the young organism, after having undergone an increase in quantity. According to this supposition therefore the germ-plasm of the parents must be found *unchanged in the germ-cells of the offspring.* If this theory were false, if the germ-plasm of the germ-cells were formed anew by the organism, perhaps from Darwin's 'gemmules' which pour into the germ-cells from all sides, it would be impossible to understand why it has not been long ago arranged that each germ-cell should receive only half the number of the ancestral gemmules present in the body of the parent. Hence the expulsion of the second polar body—assuming the validity of my interpretation—is an indirect proof of the soundness of the theory of the continuity of the germ-plasm, when contrasted with the theory of pangenesis. If furthermore, a kind of cyclical development of the idioplasm took place, as supposed by Strasburger, and if its final ontogenetic stage resulted in the re-appearance of the initial condition of the germ-plasm, we should fail to understand how any of the ancestral germ-plasms could be lost during such a course of development.

Whichever view, the latter or the theory of the continuity of the germ-plasm, be correct, in either case the male germ-cells of the young animal must contain the same germ-plasm as that which existed in the fertilized maternal egg, that is to say, they must contain all the ancestral germ-plasms of the father and the mother. Here therefore a reduction must occur, for otherwise the number of ancestral germ-plasms would be increased by one half at every fertilization. The egg-cell would furnish $\frac{1}{2}$, but the sperm-cell $\frac{2}{2}$ of the total quantity of germ-plasm present in the germ-cells of the parents. But there is no reason for believing that the reduction of germ-plasm in the sperm-cell must proceed in precisely the same way as in the egg-cell, viz. by the expulsion of a polar body. On the contrary, the processes of spermatogenesis are so remarkably different from those of ovogenesis that we may expect to find that reduction is also brought about in a different manner.

The egg-cell does not expel the superfluous ancestral germ-plasms until the end of its development, and in a form which induces the destruction of the separated portion. This is certainly remarkable, for germ-plasm is a most important substance, and although it seems to be wasted in the production of enormous quantities of sperm- and egg-cells, such waste is only apparent, and is in reality the means which renders the species capable of existence. It may perhaps be possible to prove that in this case also the waste is only apparent. Such proof would be forthcoming if it could be shown that the means by which reduction is brought about in eggs is advantageous, and therefore also, *ceteris paribus*, necessary. We see that everywhere, as far as our observation extends, the useful is also the actual, unless indeed it is impossible of attainment or can only be attained by the aid of processes which are injurious to the species. And if it be asked why germ-plasm is wasted in the maturation of egg-cells, the following may perhaps be a satisfactory answer.

Let us suppose that the necessary reduction of the germ-plasm does not take place by the separation of the second polar body, but that it happens during the first division of the first primitive-germ-cell which is found in the embryo, so that the two first egg-cells resulting from this division would already contain only half the number of ancestral germ-plasms from the father and the mother, contained in the fertilized egg-cell. In this case the main object, the reduction of the ancestral germ-plasms, would be gained by a single division, and all the succeeding nuclear divisions, causing the multiplication of these two first germ-cells, might take place by the ordinary form of nuclear division, viz. 'equal division.' But perhaps nature not only cares for this one main object alone, but also secures certain secondary advantages at the same time. In the case which we have supposed the egg-cells of the mature ovary would only contain two different combinations of germ-plasm, which we may call combinations *A* and *B*. Even if millions of egg-cells were formed, every one of them would contain either *A* or *B*, and hence (at least as far as the female pronucleus is concerned) only two kinds of individuals could arise from such eggs—viz. offspring *A′* and *B′*. All the offspring *A′* would be as similar to one another as identical twins, and the same would be true of offspring *B′*.

But if the 100th instead of the 1st embryonic germ-cell entered upon the 'reducing division,' a hundred cells would undergo this division at the same time, and thus two hundred different combinations of ancestral germ-plasm would arise, and two hundred different kinds of germ-cells would be found in the mature ovary. A still greater number of different combinations of hereditary tendencies would arise if the 'reducing division' occurred still later; but undoubtedly the diversity in the composition of the germ-plasm must be greatest of all when the 'reducing division' does not take place during the period in which the germ-cells undergo multiplication, but at the end of the entire course of ovarian development, and separately in each full-grown mature egg ready for embryonic development. In such a case there will be as many different combinations of ancestral germ-plasms as there are eggs, for, as I have shown above, it is hardly conceivable that such a complex body as the nuclear substance of the egg-cell—composed of innumerable different units—would ever divide twice in precisely the same manner. Every egg will therefore contain a somewhat different combination of hereditary tendencies, and thus the offspring which arise from the different germ-cells of the same mother can never be identical. Hence by the late occurrence of the 'reducing division' the greatest possible variability in the offspring is secured.

If my interpretation of the second polar body be accepted, it is obvious that the late occurrence of the 'reducing division' is proved. At the same time we receive an explanation of the advantage gained by the postponement of the reduction of the germ-plasm until the end of the ovarian development of the egg; because the greatest possible number of individual variations in the offspring are produced in this way.

If I am not mistaken, this argument lends additional support to the idea which I have previously propounded,—that the most important duty of sexual reproduction is to preserve and continually call forth individual variability, the foundation upon which the transformation of species is built [1].

But if it be asked whether the postponement of the 'reducing division' to the end of the ovarian development of the egg is inconsistent with the preservation of the other half of the dividing nucleus, I should be inclined to reply that a 'reducing division' of the mature egg, resulting in the production of two eggs, was probably the phyletic precursor of the present condition. I imagine that the division of the mature egg-cell—although it is now so extremely unequal—was equal in very remote times; but that for reasons of utility, connected with the specialization of the eggs of animals, it gradually became more and more unequal. It is now hardly possible to give in detail the various reasons of utility which have brought about this condition, but it may be assumed that the enormous size attained by many animal egg-cells has been especially potent in producing the change.

A careful consideration of this last point seems to me to be demanded by a comparison of the egg-cells with the male germ-cells. Just as the female germ-cells of animals are distinguished by the attainment of a large size, the male germ-cells are generally remarkable for their minute proportions. In most cases it would be physiologically impossible for a large egg-cell, rich in yolk, to attain double its specific size in order to undergo division into two equal halves and yet to remain of the characteristic size. Even without the additional difficulties imposed by the necessity for such

[1] See the preceding Essay on 'The Significance of Sexual Reproduction in the theory of Natural Selection.'

a division, all means—such as cells used as food, or the passage of food from follicular cells into the ovum, etc.—are employed in order to bring the egg-cell to the greatest attainable size. Furthermore, the 'reducing division' of the nucleus cannot take place before the egg has attained its full size, because the ovogenetic nucleoplasm still controls the egg-cell, and must be removed before the germ-plasm can regulate its development. By arguments such as these I should attempt to render the whole subject intelligible.

But the case is entirely different with the sperm-cells, which are generally minute: here it is quite conceivable that a 'reducing division' of the nuclei may take place by an equal division of the sperm-cells, occurring towards the end of the period of their formation; that is to say, in such a way that both products of division remain sperm-cells, and neither of them perishes like the polar bodies. But the other possibility also demands consideration, viz. that the reducing division may occur at an earlier stage in the development of sperm-cells. At all events, the arguments adduced above, which proved that the consequence would be a want of variability in the egg-cells, would not apply to an equal extent in the case of the male germ-cells. Among the egg-cells it may be very important that each one should have its special individual character, produced by a somewhat different composition of its germ-plasm, inasmuch as a considerable proportion of the eggs frequently developes, although this is never the case with all of them. But the production of sperm-cells is in most animals so enormous that only a very small percentage can be used for fertilization. If, therefore, e.g. ten or a hundred spermatozoa contained germ-plasm with exactly the same composition, so that, as far as the paternal influence is concerned, ten or a hundred identical individuals would result if they were all used in fertilization, such an arrangement would be practically harmless, for only one spermatozoon out of an immense number would be employed for this purpose. From this point of view we might expect that the 'reducing division' of the sperm-nucleus would not take place at the end of the development of the sperm-cell, but at some earlier period. There is no necessary reason for the assumption that this division must take place at the end of development, and without some cause natural selection cannot operate. It is, of course, conceivable that the causes of other events may also involve the occurrence of this division at the end of development; but we do not at present know of any such causes. I should not consider the influence of the specific histogenetic nucleoplasm, i.e. *the spermatogenetic nucleoplasm, to be such a* cause, because the quantitative proportions are very different from those which obtain in the formation of egg-cells, and because it is not inconceivable that the small quantity of true germ-plasm which must be present in the nuclei of the sperm-cells at every stage in their formation might enter upon a 'reducing division' with the spermatogenetic nucleoplasm, even when the latter preponderated.

As soon as we can recognize with certainty the forms of nuclear division which are 'reducing divisions,' the question will be settled as far as spermatogenesis is concerned. It has been already established that various forms of nuclear division occur at different periods of spermatogenesis. I make this assertion, not only from my own observations, but also from observations which have been made and insisted upon by others. Thus, van Beneden and Julin[1] stated in 1884 that direct and karyokinetic nuclear divisions alternate with each other in the spermatogenesis of *Ascaris megalocephala*. Again, Carnoy[2] distinctly states that the different cell-generations in the same testis may not uncommonly exhibit considerable differences as regards karyokinesis. 'This may go so far that direct and indirect division may proceed simultaneously.' Platner[3], in his excellent paper on karyokinesis in Lepidoptera, also points out that the karyokinesis of the spermatocytes is essentially different from that of the spermatogonia. According to his description, the latter form may be very well interpreted as a 'reducing division,' for no equatorial plate is formed, and the chromatin rods (or granules, as they are better called in this case) remain from the first on both sides of the equatorial plane, and finally unite at the opposite poles to form the two daughter-nuclei. Furthermore, if Carnoy has correctly observed, the form of karyokinesis which I have previously interpreted as a 'reducing division' occurs in the sperm-mother-cells—a karyokinesis in which the

[1] E. van Beneden and Julin, 'La Spermatogénèse chez l'Ascaride mégalocéphale.' Brussels, 1884.

[2] Carnoy, 'La Cytodiérèse chez les Arthropodes.'

[3] Gustav Platner, 'Die Karyokinese bei den Lepidopteren als Grundlage für eine Theorie der Zelltheilung.' Internation. Monatsschrift f. Anatomie und Histologie, Bd. III. Heft 10. Leipzig, 1886.

chromatin rods either do not divide longitudinally, or else divide in this way after they have left the equatorial plate and are proceeding towards the poles. Carnoy does not himself attach any special importance to these observations, for he only considers them as proofs that the longitudinal splitting of the loops may occur at various periods in different species—either at the equator, or on the way towards the poles, or even at the poles themselves. We cannot conclude from the author's statements whether this form of nuclear division only occurs in a single cell-generation during spermatogenesis, as it must do if it really represents a 'reducing division.' Until this point is settled, we cannot decide with certainty whether the described form of karyokinesis is to be considered as the 'reducing division' for which we are seeking. Fresh investigations, undertaken from these points of view, are necessary in order to settle the question. It would be useless to seek further support for the theory by going into further details, and by critically examining the numerous observations upon spermatogenesis which have now been recorded.

I will only mention that among the various nuclei and other bodies in different animals which have been considered by different observers as the polar bodies of the sperm-cells, or the cells which form the latter—in my opinion the paranucleus ('Nebenkern') of the 'spermatides' described by La Valette St. George[1] has the highest claim to be considered as the homologue of a polar body. But I am inclined to identify it with the first rather than the second polar body of the egg-cells, and to regard it as the histogenetic part of the nucleoplasm which has been expelled or rendered powerless by internal transformations. There are two reasons which lead me to this conclusion: first, as I have tried to show above, it is probable that the ancestral germ-plasms are not removed by expulsion, but by means of equal cell-division; secondly, my theory asserts that the histogenetic nucleoplasm cannot be rendered powerless until the close of histological differentiation.

The whole question of the details of the transformations undergone by the nucleus of the male germ-cells is not ready for the

[1] La Valette St. George, 'Ueber die Genese der Samenkörper.' Fünfte Mittheilung. Die Spermatogenese bei den Säugethieren und dem Menschen,' Archiv f. mikrosk. Anat. Bd. XV. 1878.

expression of a mature opinion. From the very numerous and mostly minute and careful observations which have been hitherto recorded, we cannot conclude with any degree of certainty when and how the 'reducing division' of the nucleus takes place, nor can we decide upon the processes which signify the purification of the germ-plasm from the merely histogenetic part of the nucleoplasm. But perhaps it has not been without value as regards future investigation that I have tried to apply to the male germ-cells the views gained from our more certain knowledge of the corresponding structures in the female, and thus to indicate the problems which now chiefly demand solution.

IV. The Foregoing Considerations applied to Plants.

It remains to briefly consider the case of plants. Obviously, the 'reducing division' of the germ-nuclei, if it takes place at all, cannot be restricted to the germ-cells of animals. There must be a corresponding process in plants, for sexual reproduction is essentially the same in both kingdoms; and if fertilization must be preceded by the expulsion of half the number of ancestral germ-plasms from the eggs of animals, the same necessity must hold in the case of plants.

But whether the process always takes place in the form of polar bodies, and not perhaps principally, or at any rate frequently, in the form of equal cell-division, is another question. It is true that polar bodies occur in numerous plants, as we chiefly know from Strasburger's researches[1]. Strasburger shows that cells are separated by division from the germ-cells, and perish. But it seems to me doubtful whether we must always regard their formation as the removal of half the number of ancestral germ-plasms rather than the histogenetic nucleoplasm of the germ-cell. It appears to me that histogenetic nucleoplasm must be present in the highly differentiated vegetable germ-cells, especially in the male cells, and also that it must be removed during the maturation of the cell, if my idea of the histogenetic nucleoplasm be accepted. It is very possible, as I have already mentioned, that there may be quite indifferent germ-cells, viz. cells which are entirely without specific histological structure, and in such cases histogenetic nucleoplasm

[1] l. c., p. 92.

would be absent; and during the maturation of such germ-cells no polar body would be formed for its removal. This view accords with the fact that polar bodies are absent in many plants. Furthermore, I am far from maintaining that in the cases where polar bodies occur, they must have the above-mentioned significance. I only wish to point out that the reduction assumed to be necessary for the nucleus of the vegetable germ-cells is not necessarily to be sought for at the close of their maturation, but perhaps even more frequently in an equal division of the germ-cells during some period of their development.

It also seems to me to be not impossible that a number of these vegetative 'polar bodies' may have an entirely different significance, viz. to perform some special function accessory to fertilization, as in the so-called 'ventral canal-cells' of the higher cryptogams and conifers. As we know that even the two polar bodies of the animal egg are not identical—although externally they are extremely similar, and although they arise in a precisely similar manner—I am even more inclined than before to consider that the very various 'polar bodies' of plants possess very different meanings.

But I do not feel justified in criticizing in detail the results of botanical investigation. I must leave the decision of such questions to botanists, and I only desire to state distinctly that a 'reducing division' of the nuclei of germ-cells must occur in plants as well as in animals.

V. Conclusions with regard to Heredity.

The ideas developed in the preceding paragraphs lead to remarkable conclusions with regard to the theory of heredity,—conclusions which do not harmonize with the ideas on this subject which have been hitherto received. For if every egg expels half the number of its ancestral germ-plasms during maturation, the germ-cells of the same mother cannot contain the same hereditary tendencies, unless of course we make the supposition that corresponding ancestral germ-plasms are retained by all eggs—a supposition which cannot be sustained. For when we consider how numerous are the ancestral germ-plasms which must be contained in each nucleus, and further how improbable it is that they are arranged in precisely the same manner in all germ-cells, and finally how incredible it is that the nuclear thread should always be divided in exactly the same place to form corresponding loops or rods,—we are driven to the conclusion that it is quite impossible for the 'reducing division' of the nucleus to take place in an identical manner in all the germ-cells of a single ovary, so that the same ancestral germ-plasms would always be removed in the polar bodies. But if one group of ancestral germ-plasms is expelled from one egg, and a different group from another egg, it follows that no two eggs can be exactly alike as regards their contained hereditary tendencies: they must all differ. In many cases the differences will only be slight, that is, when the eggs contain very similar combinations of ancestral germ-plasms. Under other circumstances the differences will be very great, viz. when the combinations of ancestral germ-plasms retained in the egg are very different. I might here mention various other considerations; but this would lead me too far from my subject, into new theories of heredity. I hope to be able at some later period to develope further the theoretical ideas which are merely indicated in the present essay. I only wish to show that the consequences which follow from my theory upon the second division of the egg-nucleus, and the formation of the second polar body, are by no means opposed to the facts of heredity, and even explain them better than has hitherto been possible.

The fact that the children of the same parents are never entirely identical could hitherto only be rendered intelligible by the vague suggestion that the hereditary tendencies of the grandfather predominate in one, and those of the grandmother in another, while the tendencies of the great-grandfather predominate in a third, and so on. Any further explanation as to why this should happen was entirely wanting. Others even looked for an explanation to the different influences of nutrition, to which it is perfectly true that the egg is subjected in the ovary during its later development, according to its position and immediate surroundings. I had myself referred to these influences as a partial explanation [1], before I recognized clearly how extremely feeble and powerless are the influences of nourishment, as compared with hereditary tendencies. According to my theory, the differences between the

[1] Weismann, 'Studien zur Descendenztheorie,' ii. p. 306, Leipzig, 1876, translated by Meldola; see 'Studies in the Theory of Descent,' p. 680.

children of the same parents become intelligible in a simple manner from the fact that each maternal germ-cell (I shall speak of the paternal germ-cells later on) contains a peculiar combination of ancestral germ-plasms, and thus also a peculiar combination of hereditary tendencies. These latter by their co-operation also produce a different result in each case, viz. the offspring, which are characterized by more or less pronounced individual peculiarities.

But the theory which explains individual differences by referring to the inequality of germ-cells, may be proved with a high degree of probability by an appeal to facts of an opposite kind, viz. by showing that identity between offspring only occurs when they have arisen from the same egg-cell. It is well known that occasionally some of the children of the same parents appear to be almost exactly alike, but such children are without exception twins, and there is every reason to believe that they have been derived from the *same* egg. In other words, the two children are exactly alike because they have arisen from the same egg-cell, which could of course only contain a single combination of ancestral germ-plasms, and therefore of hereditary tendencies[1]. The factors which by their co-

[[1] The similar conclusion that identical ova lead to the appearance of identical individuals was drawn from the same data by Francis Galton in 1875. See 'The history of the Twins, as a criterion of the relative powers of Nature and Nurture,' by Francis Galton, F.R.S., Journal of the Anthropological Institute, 1875, p. 391; also by the same author, 'Short Notes on Heredity, etc. in Twins,' in the same Journal, 1875, p. 325.

The author investigated about eighty cases of close similarity between twins, and was able to obtain instructive details in thirty-five of these. Of the latter there were no less than seven cases 'in which both twins suffered from some special ailment or had some exceptional peculiarity;' in nine cases it appeared that 'both twins are apt to sicken at the same time;' in eleven cases there was evidence for a remarkable association of ideas; in sixteen cases the tastes and dispositions were described as closely similar. These points of identity are given in addition to the more superficial indications presented by the failure of strangers or even parents to distinguish between the twins. A very interesting part of the investigation was concerned with the after-lives of the thirty-five twins. 'In some cases the resemblance of body and mind had continued unaltered up to old age, notwithstanding very different conditions of life,' in the other cases 'the parents ascribed such dissimilarity as there was, wholly, or almost wholly, to some form of illness.'

The conclusions of the author are as follows: 'Twins who closely resembled each other in childhood and early youth, and were reared under not very dissimilar conditions, either grow unlike through the development of natural characteristics which had lain dormant at first, or else they continue their lives, keeping time like two watches, hardly to be thrown out of accord except by some physical jar. Nature is far stronger than nurture within the limited range that I have been careful to assign operation controlled the construction of the organism were the same, and consequently the results were also the same. Twins derived from a single egg are identical: this is a statement which, although not mathematically proved, may be looked upon as nearly certain. But there are also twins which do not possess this high degree of similarity, and these are even far commoner than the others. The explanation is to be found in the fact that the latter were derived from two egg-cells which were fertilized at the same time. In most cases, indeed, each twin is enclosed in its own embryonic membranes, while much less frequently both twins are enclosed in the same membranes. In one point only the proof is incomplete; for it has not yet been shown that identical twins are always derived from a single egg, since such an origin, together with a high degree of similarity, could only be established as occurring together in a small proportion of the cases. We therefore see that under conditions of nutriment which are as identical as possible, *two* egg-cells develope into unlike twins, *one* into identical twins; although we cannot yet affirm that the latter result invariably follows. It is conceivable that the stimulus for the production of two eggs from one may be afforded by the entrance of two spermatozoa, but these latter, as was shown above, could hardly contain identical hereditary tendencies, and thus two identical twins would not arise. It appears indeed that some cases have been observed

to the latter.' And again, 'where the maladies of twins are continually alike, the clocks of their two lives move regularly on, and at the same rate, governed by their internal mechanism. Necessitarians may derive new arguments from the life histories of twins.'

The above facts and conclusions held for twins of the same sex, of which at any rate the majority are shown by Kleinwächter's observations to have been enclosed in the same embryonic membranes, and therefore presumably to have been derived from a single ovum; but in rarer cases the twins, although also invariably of the same sex, were marked by remarkable differences, greater than those which usually distinguish children of the same family. Mr. Galton met with twenty of these cases. In such twins the conditions of training, etc. had been as similar as possible, so that the evidence of the power of nature over nurture is strongly confirmed. Mr. Galton writes, 'I have not a single case in which my correspondents speak of originally dissimilar characters having become assimilated through identity of nurture. The impression that all this evidence leaves on the mind is one of wonder whether nurture can do anything at all, beyond giving instruction and professional training.'

The fact that twins produced from a single ovum seem to be invariably of the same sex is in itself extremely interesting, for it proves that the sex of the individual is predetermined in the fertilized ovum.—E. B. P.]

in which differences have been exhibited by twins which were enclosed in the same embryonic membranes; but nevertheless I believe that two spermatozoa are not necessary to cause the formation of twins by a single egg. We know, it is true, from the investigations of Fol[1], that multiple impregnation produces the simultaneous beginning of several embryos in the eggs of star-fishes. But several embryos and young animals are not developed in this way, for embryonic development soon ceases, and the egg dies.

The recent observations of Born[2] upon the eggs of the frog also make it very probable that a double development is produced by the entrance of two spermatozoa into the egg, but here also only monstrosities, and not twins, were produced. On the other hand, it has been shown that in birds twins may be produced from the same egg, and there is no reason for the belief that their production is due to multiple impregnation. But if it may be assumed that human twins, when identical, have been derived from a single egg, it seems to me to be extremely probable that fertilization was also effected by a single sperm-cell. We cannot understand how such a high degree of similarity could have been produced if two sperm-cells had been made use of, for we are compelled to assume that two such cells would very rarely contain identical germ-plasms.

It is most probable that the egg-nucleus coalesces with the nucleus of a single spermatozoon, but the resulting segmentation-nucleus divides together with the cell-body itself, without the occurrence of those ontogenetic changes in the germ-plasm which normally take place. The nucleoplasm of the two daughter-cells still remains in the condition of germ-plasm, and its ontogenetic transformation begins afterwards—a transformation which must of course proceed in the same way in both cells, and must lead to the production of identical offspring. This is at least a possible explanation which we may retain until it has been either confirmed or disproved by fresh observations,—an explanation which is moreover supported by the well-known process of budding in the eggs of lower animals.

[1] Fol, 'Recherches sur la fécondation et le commencement de l'hénogénie.' Genève, Bâle, Lyon. 1879.

[2] Born, 'Ueber Doppelbildungen beim Frosch und deren Entstehung.' Breslauer ärztl. Zeitschrift, 1882.

VI. Recapitulation.

To bring together shortly the results of this essay:—the fundamental fact upon which everything else is founded is the fact that *two* polar bodies are expelled, as a preparation for embryonic development, from all animal eggs which require fertilization, while only *one* such body is expelled from all parthenogenetic eggs.

This fact in the first place refutes every purely morphological explanation of the process. If it were physiologically valueless, such a phyletic reminiscence of the two successive divisions of the egg-nucleus must have been also retained by the parthenogenetic egg.

In my opinion the expulsion of the first polar body implies the removal of ovogenetic nucleoplasm when it has become superfluous after the maturation of the egg has been completed. The expulsion of the second polar body can only mean the removal of part of the germ-plasm itself, a removal by which the number of ancestral germ-plasms is reduced to one half. This reduction must also take place in the male germ-cells, although we are not able to associate it confidently with any of the histological processes of spermatogenesis which have been hitherto observed.

Parthenogenesis takes place when the whole of the ancestral germ-plasms, inherited from the parents, are retained in the nucleus of the egg-cell. Development by fertilization makes it necessary that half the number of these ancestral germ-plasms must be first expelled from the egg, the original quantity being again restored by the addition of the sperm-nucleus to the remaining half.

In both cases the beginning of embryogenesis depends upon the presence of a certain, and in both cases equal, quantity of germ-plasm. This certain quantity is produced by the addition of the sperm-nucleus to the egg requiring fertilization, and the beginning of embryogenesis immediately follows fertilization. The parthenogenetic egg contains within itself the necessary quantity of germ-plasm, and the latter enters upon active development as soon as the single polar body has removed the ovogenetic nucleoplasm. The question which I have raised on a previous occasion—'When is the parthenogenetic egg capable of development?'—now admits of the precise answer—'Immediately after the expulsion of the polar body.'

From the preceding facts and considerations the important conclusion results that the germ-cells of any individual do not contain the same hereditary tendencies, but are all different, in that no two of them contain exactly the same combinations of hereditary tendencies. On this fact the well-known differences between the children of the same parents depend.

But the deeper meaning of this arrangement must doubtless be sought for in the individual variability which is thus continuously kept up and is always being forced into new combinations. Thus sexual reproduction is to be explained as an arrangement which ensures an ever-varying supply of individual differences.