

*The Chromosome Cycle in Coccidia and Gregarines.*

By CLIFFORD DOBELL and A. PRINGLE JAMESON.

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Despite the large amount of work which has already been devoted to the study of the Coccidia and Gregarines, very little indeed is known definitely about the behaviour of the chromosomes in these Protozoa. Not only has the chromosome cycle been left uninvestigated and undescribed in the majority of these organisms which have hitherto been studied, but the very existence of chromosomes in the nuclear divisions at many stages in the life-history of certain forms has even been denied; and the most contradictory and unsatisfactory accounts have been given of that most important phase in the life-cycle of the chromosomes—the phase of meiosis, or reduction.

In order to fill up this gap in our knowledge of the Sporozoa, we have made—during the last few years—a very detailed study of the chromosomes of a coccidian and a gregarine. One of us (C. D.) has investigated the coccidian\* *Aggregata eberthi* Labbé, whilst the other (A. P. J.) has studied the gregarine *Diplocystis schneideri* Kunstler. Careful investigation of these two organisms has shown that the nuclear divisions at all stages in the life-histories are mitotic, and that the chromosome numbers are remarkably constant.

As our results are, we believe, quite definite and conclusive, and as the publication of them in full is likely to be unavoidably delayed for some time, we think it desirable to place them on record. Complete accounts of the life-histories of *Aggregata* and *Diplocystis* we hope to publish separately elsewhere. We shall here deal only with the essential facts which we have established concerning the chromosomes of these two forms.

1. *The Chromosomes of Aggregata eberthi.*

The life-history of this coccidian comprises a sexual generation which takes place in the body of a cuttle-fish (*Sepia officinalis*), and an asexual generation in the body of a crab (*Portunus depurator*). These generations are of the usual coccidian type. In the sexual cycle, male and female individuals (“microgametocytes” and “macrogametocytes”) are formed, which give rise to microgametes and macrogametes respectively. Each of the latter is fertilized by one of the former; and after the union of the two nuclei, the zygote nucleus divides many times to form the nuclei of the numerous

\* That this organism is really a coccidian and not a gregarine has already been shown (cf. Dobell, 1914).

sporoblasts. The uninucleate sporoblasts are then converted into spores, within each of which three uninucleate sporozoites are finally formed by further divisions of the nucleus and differentiation of the cytoplasm. In the asexual cycle, the sporozoites, after escaping from the spores, grow into large schizonts. By repeated divisions of the nucleus, each schizont becomes multinucleate, and finally breaks up into a very large number of uninucleate merozoites. When the latter leave the crab and enter the body of the cuttlefish, they grow into the male and female individuals of the sexual cycle—thus completing the life-history.\*

We may begin the history of the chromosomes with a description of these bodies in the male parasite. During the period of growth, the nucleus passes through a series of very complex stages which need not be described here. At the end of this period the first nuclear division takes place. This division begins as an ordinary mitosis, but ends as a multiple mitosis of a peculiar type. In the prophase, six filamentar chromosomes are formed from the spireme. At diakinesis they can be clearly seen and counted. They consist (fig. 1, A) of one very long chromosome (*a*) and one very short chromosome (*f*), the remaining four (*b-e*) forming a regular series of intermediate sizes. During the period of the first division, the karyosome disintegrates and disappears. It plays no part in the formation of the chromosomes.

As the chromosomes pass on to the equatorial plate of the first spindle, they shorten and thicken until they become almost spherical. They preserve, however, at this and all subsequent stages, their characteristic size-relations to one another (fig. 1, B, *a-f*). At the metaphase they divide by constriction, a daughter-group of six differentiated chromosomes passing to each pole of the spindle (fig. 1, C). In the late anaphases the chromosomes again become filamentar. The asters at the poles of the spindle divide many times in succession, and each time all the chromosomes split longitudinally. A complicated polyester figure is thus formed, from which the chromosomes finally emerge in groups of six (fig. 1, D, *a-f*). Each group enters into the formation of a resting nucleus at the periphery of the organism. After this first multiple division, the nuclei divide many times in succession by ordinary bipolar mitosis—the typical set of six chromosomes being recognisable at each division. The smallest nuclei finally formed enter into the microgametes. No halving of the chromosomes takes place, therefore, in the formation of these. Each microgamete nucleus receives a typical set of six chromosomes, like those formed for the first division.

The macrogamete is formed, in the typical coccidian manner, by each female individual being transformed into a single gamete. The nucleus of

\* See Siedlecki (1898), Léger and Dubosq (1908), Dobell (1914).

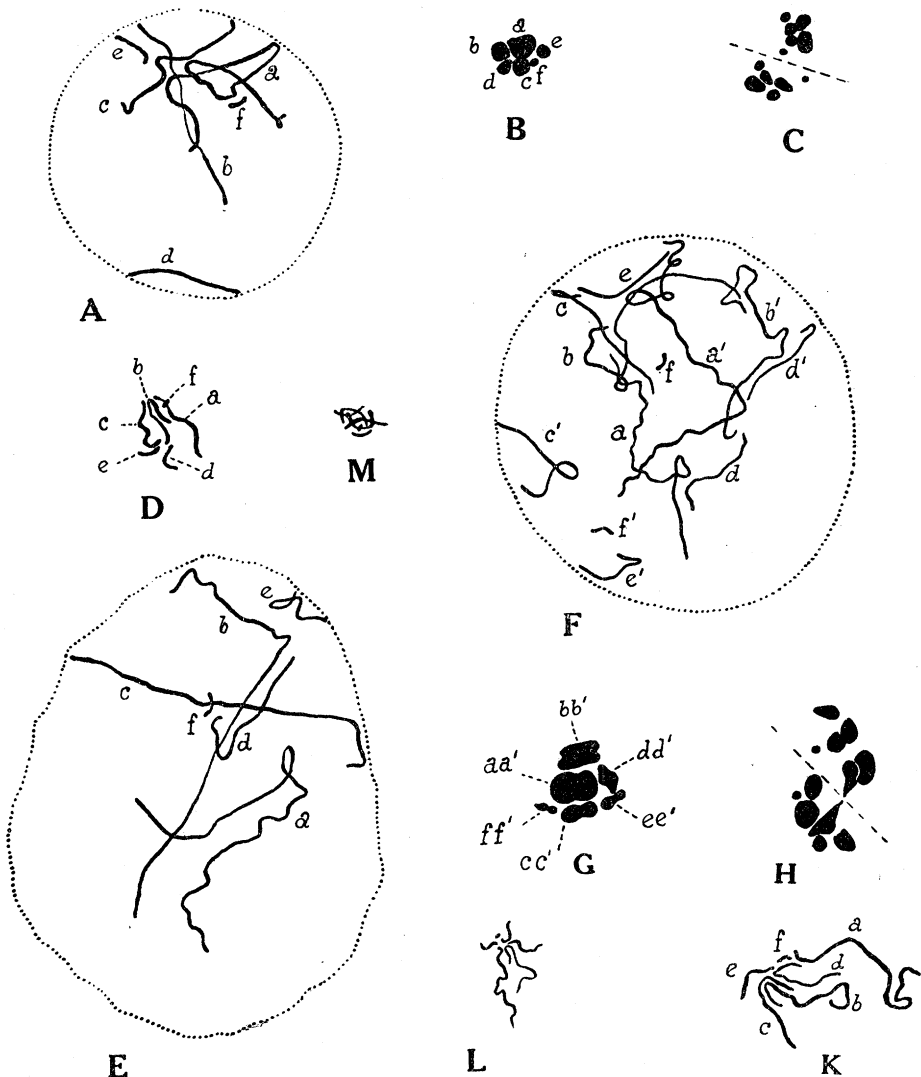


FIG. 1.—Chromosomes of *Aggregata eberthi*.

The figures are tracings (of chromosomes only) from detailed drawings made with the camera lucida at a magnification of 2500 diameters. The letters *a-f* (or *a'-f'*) indicate throughout the six chromosomes forming the haploid group, or typical chromosome complex, of *Aggregata*. The chromosomes are lettered consecutively in order of magnitude—from *a* (the largest) to *f* (the smallest).

A. Nucleus of male—prophase of first division. B. Equatorial plate—first division of male. C. Early anaphase groups at first division in male. D. Chromosome group entering into resting nucleus—end of first (multipolar) division of male. E. Chromosomes in macrogamete nucleus before fertilization. F. Chromosomes in zygote nucleus—prophase of first division. G. Double (paired) chromosomes on equatorial plate—first spindle of zygote nucleus. H. Early anaphase groups—first spindle of zygote. K. Chromosome group entering into resting nucleus—end of first (multipolar) division of zygote. L. Chromosome group—anaphase of later division (bipolar) of zygote. M. Chromosome group on equatorial plate of second division in spore.

the female passes through a complex series of stages similar to those occurring in the male; and these, likewise, culminate in the formation of chromosomes, though no nuclear division takes place before fertilization. Here, again, the chromosomes can be clearly counted, and consist of six filaments differing in size from one another (fig. 1, E,  $a-f$ ) exactly as in the male. The karyosome breaks up and disintegrates; and, as in the male, plays no part in forming the chromosomes.

Before fertilization the chromosomes break up within the nucleus of the macrogamete. The microgamete then enters, and a series of very complex nuclear changes ensues. These include the formation of a so-called "fertilization spindle" characteristic of the Coccidia. At the end of these changes the zygote nucleus divides—the division beginning like an ordinary mitosis and then becoming multiple, as in the first division in the male. The behaviour of the chromosomes during this division is very remarkable. From the spireme 12 chromosomes are formed, which can be clearly counted at diakinesis (fig. 1, F). They consist of two homologous sets of six chromosomes each—each set ( $a-f$ ,  $a'-f'$ ) consisting of six filaments differentiated as regards length from one another. The two sets clearly represent the two groups of chromosomes which the zygote nucleus has received from the male and female pronuclei.

When the achromatic spindle has been formed for the first division of the zygote nucleus, the 12 chromosomes pass on to its equator. As they do so they shorten and thicken until they become almost globular. At the same time they associate in homologous pairs— $a$  with  $a'$ ,  $f$  with  $f'$ , etc.—so that six bivalent or double chromosomes finally come to lie on the equatorial plate (fig. 1, G). At the metaphase, disjunction of the temporarily united chromosomes takes place, without any splitting of the individual chromosomes; so that a group of six differentiated chromosomes passes towards each pole of the spindle (fig. 1, H). The rest of this division resembles the first division in the male. The asters divide, and the chromosomes become filamentar and split lengthwise. From the polyaster figure so formed the chromosomes emerge finally in sets of six (fig. 1, K) and enter into the formation of as many resting nuclei as there are sets of chromosomes.

The subsequent nuclear divisions of the zygote all take place by ordinary bipolar mitosis. At each division a typical set of six chromosomes (fig. 1, L) is present—one such set entering, therefore, into the nucleus of each sporoblast. A similar set of six chromosomes can be counted at each of the two mitotic divisions which take place within the spore to form the sporozoite nuclei (fig. 1, M).

During the development of the sporozoite into a schizont, the nucleus

passes through a long series of stages closely similar to those which occur in the young sexual forms. We have not been able to study the nuclear divisions of the schizont in such great detail as we have those of the sexual parasites: but it is evident from our results, taken together with those of Léger and Duboscq (1908), who have previously described these stages, that the number of chromosomes is six throughout the entire asexual cycle. The first division of the schizont nucleus is by multiple mitosis, the later divisions by ordinary mitosis into two—as in the sexual forms. The typical size-relations of the six chromosomes are probably recognisable throughout. (We have not been able to study every division, but those which we have all appear quite typical.)

From the foregoing description, it will be clear that in *A. eberthi* the chromosomes are six in number at every nuclear division in the life-history with one exception. This is the division of the zygote nucleus immediately succeeding fertilization. There are here 12 chromosomes, which become halved to six in the course of this division. Reduction thus occurs immediately after fertilization—not during gametogenesis. The six chromosomes must be regarded as representing the haploid number—the diploid number (12) being present in the zygote nucleus only, while its division is a reduction division. Since the haploid number (6) occurs in both the sexual and the asexual cycle, there is thus no differentiation in respect of chromosome number in the two generations.

## 2. *The Chromosomes of Diplocystis schneideri.*

The life-history of *D. schneideri* is comparatively simple. It is passed in a single host—a cockroach. We have studied it chiefly in *Periplaneta americana*, but have found it also in *Stylopyga orientalis*. At a very early stage in their development the parasites unite in pairs in the gut wall of their host, and then fall into the body cavity. After a considerable growth period in the associated condition, they form gametes. The nucleus of each member of the pair gives rise, by repeated mitoses, to a great number of very small nuclei—each of which finally becomes the nucleus of a gamete. The partition separating the two individuals (gamonts) has by this time disappeared; and the gametes, when fully formed, fuse in pairs. The nucleus of the zygote (sporoblast) gives rise to eight daughter nuclei which become the nuclei of the eight sporozoites formed within each spore. The sporozoites develop within another cockroach into body-cavity parasites once more, and the life-cycle is thus completed.

Although the mitotic figures at certain stages are very small, the behaviour

of the chromosomes can be followed with comparative ease all through the life-history.

The gamont nucleus gives rise to a small achromatic spindle, on to the equatorial plate of which three tiny chromosomes pass. These are formed from a little vesicular karyosome which lies near the spindle, and which is all that remains of the originally abundant chromatin of the nucleus. They are at first filamentar, but shorten and thicken until they become globular on the equatorial plate (see fig. 2, A). Each chromosome divides by constriction into two at the metaphase (fig. 2, B), and three daughter chromosomes pass to each pole of the spindle (fig. 2, C). Of the three chromosomes two are approximately equal in size and round in outline, while the third is slightly larger and ovoid.

In the second mitotic division the chromosomes are again present in the form of three globules. At the third, however, they are stumpy rods. But at the next division, and those immediately following it, they are filamentar. They split longitudinally at the metaphases (fig. 2, D) and pass as filaments to the poles of the spindles (fig. 2, E). A definite size-relation is usually visible—one chromosome being long, one somewhat shorter, and the third intermediate (*cf.* fig. 2, E).

During later divisions the nuclei become smaller, and travel to the periphery of the organism. At the same time the chromosomes become shorter and more stumpy (fig. 2, F). The nuclei multiply still further at the periphery. When they have become very numerous and small—immediately previous to gamete formation—the chromosomes are seen to be once more globular, and to divide by constriction (fig. 2, G). The gametes are now formed, and fuse in pairs.

When the nucleus of the zygote (sporoblast) is about to divide, its chromatin breaks up into numerous granules, which subsequently unite to form a spireme. The spireme is at first an open one, but it afterwards contracts into a somewhat tangled knot towards one side of the nucleus. The knot then opens out, and the spireme segments into six filamentar chromosomes (fig. 2, H). Of these two are long, two short, and two intermediate. At the division which now takes place these separate into two homologous sets of three each, which pass to the daughter nuclei (fig. 2, K). The latter rapidly divide twice in succession, thus giving rise to the eight sporozoite nuclei. Three chromosomes are visible at each of these divisions: but at the second they are short rods (fig. 2, M), and at the third globular (fig. 2, N).

It will thus be seen that the number of chromosomes in all the nuclear divisions of *D. schneideri* is, with one exception, three. At the first mitosis

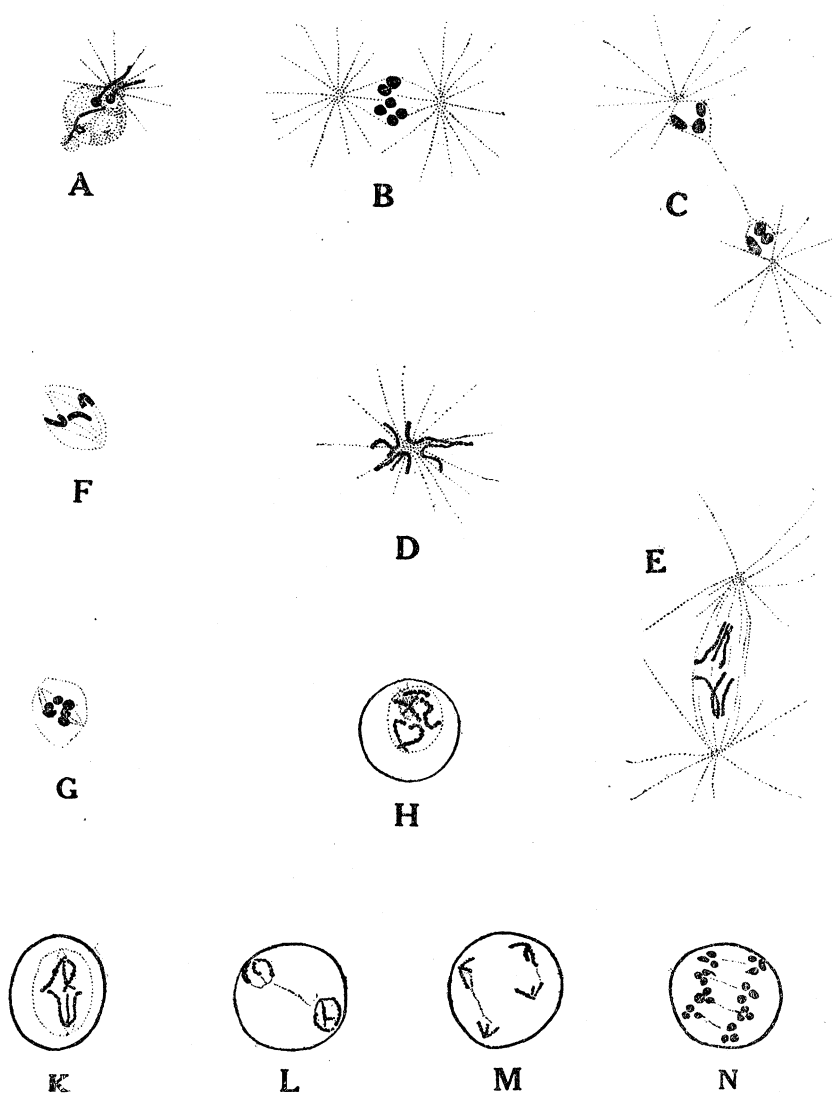


FIG. 2.—Chromosomes of *Diplocystis schneideri*.

The figures are tracings from drawings made with the camera lucida at a magnification of 2500 diameters. Only the chromosomes are exactly figured; the other structures are semi-diagrammatic.

A. Early stage in first division of gamont nucleus. Chromosomes lying upon residual karyosome. The aster is also indicated. B. Metaphase of first division. C. Anaphase (late) of first division. D. Metaphase of later division, viewed from pole. E. Anaphase of later division. F. Equatorial plate stage of early peripheral division. G. Early anaphase of late peripheral division, immediately previous to gamete formation. H. Prophase (diakinesis) of first division in zygote (sporoblast). K. Anaphase of first division in zygote (spore). L. Early telophase of first division in spore. M. Anaphase groups at second division in spore. N. Anaphase groups at third division in spore.

in the spore, six chromosomes are formed by the segmentation of the spireme thread. These separate into two homologous groups of three each, which clearly represent the two sets of chromosomes derived from the two gamete nuclei which united to form the nucleus of the zygote. This first division in the spore—immediately following fertilization—must, therefore, be regarded as a reduction division.

The haploid number of chromosomes in *D. schneideri* is therefore three, and occurs in all the nuclear divisions of the gamont, and in all the spore divisions except the first. The diploid number is six, and is found at only one division in the whole life-cycle—the first sporal division, immediately following fertilization. Reduction thus occurs directly after fertilization, and not during gametogenesis.

#### *Conclusions.*

Although it is not yet possible to make any definite statement concerning the chromosome cycle of the Coccidia and Gregarines in general from the two individual instances which we have investigated, nevertheless we believe that certain justifiable conclusions can be drawn from the results of our work. We will briefly indicate what these are.

Both in *Aggregata* and in *Diplocystis* the chromosomes are so remarkably constant throughout the entire life-cycle that we believe this must be the case in other Coccidia and Gregarines also. The apparent exceptions, in which “amitotic” and “chromidial” nuclear divisions have been described, are probably in every instance referable to abnormal or degenerate conditions in the organisms studied, or to faulty cytological technique. We now know this to be true at least of *Aggregata* and *Diplocystis* and other Sporozoa which we have ourselves studied.

So far as we are aware, the chromosomes have not previously been exactly counted in any coccidian. “Reduction” has, however, been described in a number of forms (*cf.* Schaudinn, 1900; Siedlecki, 1898; etc.); but it is evident that this “reduction” has nothing whatever to do with reduction properly so called, *i.e.* with halving of the chromosome number. The “reduction” and “épuration nucléaire” described during gametogenesis are merely stages in the disintegration and elimination of the karyosome, and have nothing whatever to do with the formation of the gamete nuclei. These “reductions” have probably been so termed because they occur during gametogenesis; but in *Aggregata* at least—and probably in other coccidia—the chromosomes are present in the reduced number throughout this process.

In *Cyclospora* two “reduction-divisions” have been described (Schaudinn, 1902) in the maturation of the macrogamete. No chromosomes were observed,



however, and it seems to us probable that the phenomena described were misinterpreted. No "polar nuclei" like those described in *Cyclospora* are formed by *Aggregata*; and from the study of the chromosomes, it is clear that the occurrence of reduction-divisions at this stage is improbable, as the haploid number of chromosomes is already present.

In Gregarines the chromosomes have been more fully studied than in Coccidia. Their number has been counted in several forms, and it is significant that in some of these an odd number has been found. In addition to *D. schneideri* with three chromosomes, we may note *Echinomera hispida* with five (Schellack, 1907), *Nina gracilis* with five (Léger and Duboscq, 1909), and *Gregarina ovata* with three (Schellack, 1912). It is somewhat surprising that what is probably the correct explanation of this uneven number—namely, that it is the haploid number—has not previously been given. It has been suggested (Schellack, 1907; Léger and Duboscq, 1909) that the odd number is due to the presence of an "accessory" or "unpaired" chromosome—it being assumed that the odd number is the diploid number, and that reduction occurs in gametogenesis. We can find no good evidence to prove that a true "accessory" chromosome is really present in any of the organisms in which it has been described. The authors admit that the supposed "accessory" is characterized chiefly by its size—which is no criterion—though in *G. ovata* it is hardly, if at all, distinguishable from the other chromosomes (Schellack, 1912). It is also stated that this "accessory" or "axial" chromosome helps to form the karyosome of the resting nucleus; though in *G. ovata* a karyosome is not formed. We believe that the "axial" chromosome possesses no special significance, the not infrequent presence in Gregarines of an odd number of chromosomes being explained by the fact that it represents, as in *D. schneideri*, the reduced number.

There has already been a good deal of speculation concerning "reduction" in the Gregarines, though it rests upon a very small basis of fact. In *G. ovata*, Paehler (1904) and Schnitzler (1905) have described the formation of a "polar body" by an unequal division of the nucleus during the "maturation" of the gamete. Léger and Duboscq (1909) have described a similar "reduction" in *G. munieri*, though two "reduction-nuclei" are said to be formed in this case. Whatever the correct interpretation of these "polar nuclei" may be, it is important to notice that a halving of the chromosome number during their formation has in no case been demonstrated. Until this has been done, we cannot regard these observations as indicating that a real nuclear reduction occurs in this way during gametogenesis in the organisms studied. As, furthermore, the number of chromosomes present in these cases previous to the so-called "reduction" appears to be an odd one, it is difficult

to imagine how the halving of the number could occur. It appears to us highly probable that, as in *D. schneideri*, no reduction occurs at these stages in any of these organisms.

There are only two cases of alleged reduction during gametogenesis in Gregarines in which chromosome counts have been made. Recently Trégouboff (1914) claims to have established a reduction of this sort in *Stenophora juli*. The chromosomes are said to be reduced from four to two by an unequal nuclear division, which takes place in the macrogametes either before conjugation, or during conjugation, or after conjugation. It is impossible to examine Trégouboff's statements in detail here; but a careful investigation of his figures and descriptions leads us to believe that the phenomena observed have been misinterpreted. The evidence presented at least is far from establishing his contentions.

The only other case in which reduction is said to occur during gametogenesis, and in which the chromosomes have been counted, is that of *Monocystis rostrata*, described by Mulsow (1911). Here the chromosomes are stated to be reduced from eight to four at the nuclear division immediately preceding gamete formation. This account does not agree with those of others in which "polar nuclei" have been described, and is very different from what we have found in *Diplocystis*. From a careful examination of Mulsow's work we are at present inclined to believe that he was in reality dealing with two different species of *Monocystis*—one with eight chromosomes, the other with four; and that no reduction occurs during gametogenesis. Unfortunately, the division which we regard, on analogy with *D. schneideri*, as probably the reduction division—namely, the first nuclear division in the spore—is not properly described by Mulsow. The investigation of this division is, however, of the utmost importance for the establishment of his claims. If Mulsow's interpretations are correct, we shall be forced to conclude that the chromosome cycle of *M. rostrata* is fundamentally different from that of *D. schneideri*, and from that which we believe to occur in other Gregarines and in Coccidia.

We consider that neither of the above cases—*Stenophora* or *Monocystis*—is perfectly satisfactory, considered entirely on its own merits. And this is not to be wondered at if the chromosome cycle of the Gregarines in general is, as we believe, like that which we have found in *D. schneideri*. The chromosomes are here present in their haploid number during gametogenesis, and accordingly their reduction is not to be expected at this stage.

It is clear that the chromosome cycle of *Aggregata* agrees, in principle, with that of *Diplocystis*. In both forms the haploid number of chromosomes is found in every nucleus throughout the entire life-history, with the single

exception of the zygote nucleus. This is a diploid nucleus, containing two haploid groups of chromosomes derived from the two gamete nuclei which entered into its formation. The division of this nucleus is, in both *Aggregata* and *Diplocystis*, a reduction division, which reduces the diploid number to the haploid once more. From the point of view of the chromosomes, it is thus clear that the first division of the sporont nucleus of *Aggregata* is not homologous with the first division of the "sporont" (gamont) nucleus of *Diplocystis*, but with the first division of its spore nucleus—since these are the divisions during which the chromosome numbers are halved.

A chromosome cycle such as we have found in the two members of the Sporozoa here described has not, we believe, been previously demonstrated in any of the Protista. It has, however, been supposed—though not proved—to occur in some forms.

We regard the supposition that reduction occurs during gametogenesis in the Coccidia and Gregarines as an incorrect analogy drawn from other animals. The Coccidia and Gregarines are a very homogeneous group of organisms. And the chromosome cycle is, as a rule, so constant a character in any natural group of animals or plants, that we find every reason to believe at present that the chromosomes of the Sporozoa\* generally will be found—when more fully studied—to behave like those of *Aggregata* and *Diplocystis*. There is at least, we believe, no evidence from other Coccidia, and but little from other Gregarines, which can be urged with any cogency against this view.

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\* By "Sporozoa" we mean the so-called Telosporidia only. We regard the "Neosporidia" as totally unrelated organisms.

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*Experiments on the Restoration of Paralysed Muscles by Means of Nerve Anastomosis. Part III.—Anastomosis of the Brachial Plexus, with a Consideration of the Distribution of its Roots.\**

By ROBERT KENNEDY, M.A., D.Sc., M.D., St. Mungo Professor of Surgery in the University of Glasgow.

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(Abstract.)

In two former communications† experiments on anastomosis of nerves as applied to the facial nerve in the dog and in the monkey and to the limb nerves in the dog were published. The series of experiments presented in this paper has reference to anastomosis as applied to the roots of the brachial plexus. In the brachial plexus, as compared with the nerves in the limb distal to the plexus, experiments of this nature require special consideration, as in the former the fibres to the same muscle or muscles usually pass in more than one root, and consequently any conclusions from the operation of anastomosis are liable to be fallacious, unless this is taken into account.

It is necessary, therefore, to consider in these experiments on anastomosis also the question of the distribution of the roots of the plexus.

In two of the experiments only a single root, namely, the fifth or the sixth cervical, was permanently cut off from its centres and attached to a neigh-

\* The expense of this research has been defrayed by a Government Grant from the Royal Society.

† 'Phil. Trans.,' B, vol. 202, p. 93 (1911), and vol. 205, p. 27 (1914).