before it the abdominal wall; if this movement does not occur, the inference is that the diaphragmatic contraction does not take place, because it is adherent to the pericardium, which maintains an almost constant position, owing to its relations to the root of the lungs and the aorta and pulmonary artery, though its junction to the central tendon of the diaphragm causes it to descend a little when the diaphragm descends.

The impulse in these cases is often very diffused and the apex of the heart can generally be felt in the same position, not moving if the patient changes his position and lies on either side, the whole pericardium being anchored and fixed by adhesions. The limitation of diaphragmatic movement has a very important bearing on the liver. Physiologically it is compressed by the descent of the diaphragm, and in its turn presses on the vena cava, so that the blood from the hepatic veins is pumped up through the inferior vena cava into the right auricle. If the diaphragm does not act, this free passage of blood does not take place; it is dammed up in the liver, which becomes swollen, and, as in the case of H. S., may be felt as low down as the umbilicus. If, therefore, in a patient who has been known or suspected to have adherent pericarditis we find such enlargement, and this is not due to cirrhosis, we may feel pretty confident that the diaphragm is adherent to the pericardium, especially if the diaphragm moves less than normally, and the anterior wall of the abdomen does not protrude with inspiration.

The vena cava passes between the right and central lobes of the central tendon of the diaphragm, and it is more than likely that its shape may be altered and its lumen diminished by an increase of the connective tissue of the tendon, due to inflammatory proliferation.

This leads me to express the hope that it may be possible, by means of removal of this pressure on the vena cava, to still further increase the relief that the present operation of cardiomyolysis has rendered. It would, of course, necessitate a second operation on the right side, and it may indeed be impossible, as the vena cava is deep in the thorax and has very intimate relations with the phrenic nerve. Mr. Billington, resident pathologist at the General Hospital, has been kind enough to make a number of investigations for me on the relations of the inferior vena cava in the thorax, and finds that there is a very appreciable length of the vein, an inch or more, which is to be seen in the thorax between the points of piercing the diaphragm and entering the heart.

All the older textbooks speak of the vein as passing through the diaphragm and entering the pericardium immediately. This is certainly not the case, as the vein can be distinctly seen as stated for $\frac{3}{4}$ in. before it pierces the pericardium; the rest of it, comprising about $\frac{1}{2}$ in., is within the pericardium and partially covered by the serous sac. The later textbooks speak of and illustrate this fact.

It is just this first part which may be affected by adhesions of the right pleura, which are very apt to occur in cases of anterior mediastinitis, secondary to pleurisy or pericarditis. It is to the possibility of dealing with this condition that I venture to draw the attention of surgeons. The vena cava, when it leaves the diaphragm, is extra-pericardial and is covered by fibrous tissue; what this fibrous covering is seems doubtful. It may be a derivative from the diaphragm or from the fibrous bag of the pericardium, or be independent of both. It is not covered by the pericardial serous sac until about $\frac{1}{2}$ in. before it enters the right auricle. This $\frac{3}{4}$ in. of vena cava, which has as yet no connexion with the serous sac of the pericardium, is therefore very likely to be affected by any proliferation of connective tissue, such as occurs in mediastinitis; but it is equally obvious that adhesions of the two layers of the serous sac—namely, that overlying the heart muscle and that lining the fibrous bag of the pericardium—cannot affect this part at all.

It is possible, therefore, to understand why internal adhesions of the pericardium may occur without inducing any signs of pressure on the cava, such as obstruction to the entrance of the blood into the right auricle, and consequent increase in size of the liver. When such signs occur it would follow that we may assume that external adhesions have been formed. This interpretation, if correct, would explain why sometimes chronic pericarditis may exist for years without producing any symptoms,

until gradually those of progressive myocardial degeneration assert themselves, and, on the other hand, why very rapid signs of heart failure without general valvular lesions may probably be found to be dependent on external pericardial adhesions.

It is in this latter type of case that cardiomyolysis may be expected to prove useful.

The anatomical conditions of the vena cava superior as regards its relation to the pericardium are exactly like those of the vena cava inferior, but the symptoms associated with pressure upon it will be those of delay of the venous circulation in the face and neck.

Cyanosis, which was so marked in the case of H. S., disappeared at once after the operation.

No attempt was made in this case to look for adhesions to the diaphragm or the veins, so it is clear that the beneficial result was due to the increased freedom of contraction from removal of the ribs, and by separation of the adhesions between them and the pericardium.

AN INTRACELLULAR PARASITE DEVELOPING INTO SPIROCHAETES,

FOUND BY THE JELLY METHOD OF IN VITRO STAINING IN SYPHILITIC LESIONS AND IN THE CIRCULATING BLOOD DURING THE SECONDARY STAGES OF THE DISEASE.*

(With Special Plate.)

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1. An Intracellular Parasite of Guinea-pigs developing into Spirochaetes.

By the jelly method of *in vitro* staining,^{1,2} certain peculiar inclusions found embedded within the mononuclear leucocytes of the blood of guinea-pigs and known as Kurloff's bodies were shown to be parasites.¹⁰ These same bodies, if examined by the usual methods involving drying and fixation, merely appear as opaque semi-stained "splodges," and therefore can be easily passed over as artefacts. Hence their true nature was not recognized until the jelly method was employed. The method enabled the development of this parasite, which was named *Lymphocytozoon cobayae*, to be demonstrated. It showed how the chromatin within the inclusion becomes formed into spirochaete-like bodies, and how, after the inclusion has burst, the spirochaetes swim freely in the blood. They can then be seen readily by the dark-ground illuminator as motile filaments swimming in the blood of the guinea-pigs. In addition to this, amoeboid forms were also observed. In the description of this parasite, the suggestion that these spirochaete forms might be microgametes (male element or spermatozoon) was quoted¹; and the observations were made that the parasite produces anaemia, and that many of the infected guinea-pigs—a large percentage of guinea-pigs bought in England are infected—exhibited single or multiple white patches or tumours within the substance of the liver and spleen.

2. The Histology of the White Liver Tumours of Guinea-pigs.

Microscopic sections of these gumma-like tumours of the liver and spleen of guinea-pigs were then furnished me,³ and these showed that the tumours contained masses of mononuclear cells, the majority of which exhibited the cell inclusions known as Kurloff's bodies.

3. The History of the Parasite of Guinea-pigs.

Kurloff³ thought that the cell-inclusions were vacuoles; another⁴ suggested "Secretstoffe"; while again others^{5,6} claimed their parasitic nature. More recently it has been maintained⁸ that they belong to the Chlamydozoa or Cytoryctes. The supposition of their parasitic nature, however, led to conjectures as to the mode of transmission, and food, ectoparasites, and contact have been suspected. But I have already mentioned that by means of the jelly method these Kurloff bodies have now been proved to be living parasites; and observations point to the transmission of these parasites from guinea-

* Received October 11th, 1912.

pig to guinea-pig being effected at coitus, and also transferred from mother to offspring.*

4. An Intracellular Parasite of Earthworms developing into Spirochaetes.

J. W. Cropper⁹ found similar cell inclusions within the fixed mononuclear cells of the vesiculae seminales of earthworms; these very closely resemble in appearance the parasites found in the blood of guinea-pigs. By the jelly method Cropper demonstrated the development of these earthworms' parasites through precisely similar stages to those undergone by Kurloff's bodies into free swimming spirochaetes (seen readily by the dark-ground illuminator) which are to be found in the seminal fluid of the infected earthworms.

5. An Intracellular Parasite developing into Spirochaetes found in Human Syphilis.

A review of the foregoing facts led to the examination of the sores of syphilitic patients by the jelly method, and cell inclusions similar to Kurloff's bodies were found at once. The first case examined suffered from a non-ulcerated Hunterian chancre, which was pricked with a needle and the blood placed upon a jelly and examined. Several of the mononuclear leucocytes exhibited distinct cell inclusions, each one of which was embedded within the cell cytoplasm itself, surrounded by a cell wall, and having a distinctly staining chromatin mass within its interior. In the same specimen round or pear-shaped bodies were seen lying free between the blood corpuscles; these were similar in shape, size, staining reactions, presence of chromatin nucleus, cytoplasm, granules, as those seen included within the cells.

Since then 143 cases of primary and secondary syphilis have been examined by this method, and the intracellular and extracellular bodies have been found in every case. They have been observed in the chancres, in the "hard shotty" non-suppurating glands removed aseptically, in the blood from the maculae and condylomata, in the ulcerated tonsils, and in the peripheral (finger) blood. And in consequence of the number which have been observed, their resemblance to the parasites of guinea-pigs and earthworms described above, and because of their characteristic appearance when seen stained by the jelly method, their parasitic nature is evident; and now various phases of their development have been demonstrated, and the mode of the production of the spirochaete stage seen. The interpretation which I place upon the phases of the development observed in this parasite are as follows: The small extracellular round or pear-shaped bodies, numerous in the chancres and glands (homologous to the free, amoeboid forms of *Lymphocytozoon cobayae*), become included within the cytoplasm of the leucocytes, epithelial and other cells. Here they encyst themselves in a cell wall, grow, and their chromatin undergoes division and subdivision.

These included bodies are very frequently seen in the depths of the chancres, glands, and sores. They are found multiple or single embedded within the cytoplasm of the large mononuclear cells; and as the parasites grow, the nucleus of the cell becomes distorted and squeezed. The chromatin within the parasite stains (on the jelly to be described) purple—each chromatin mass having in its centre a well marked very deeply staining dot. There may be one chromatin mass within the inclusion, or there may be as many as twelve, according to the size and stage of the development of the parasite. Each of these chromatin masses is either round or pear-shaped, and some of them may be seen apparently in the act of subdivision. In the full-grown parasite commonly found included within the large mononuclear cells squeezed from the deeper parts of the chancres, or found in the glands removed by operation, each individual chromatin mass will be seen to resemble the free, round or pear-shaped forms already described. In fact, the cell inclusion can be made to burst on the jelly by pressure on the cover-glass, and the contained young parasites extruded.

But there is another phase to be recorded. In some instances the chromatin mass within the cell inclusion gives rise, while still within its host-cell and when it has reached full size, to several minute purple-staining, curled and twisted spirochaete-like tails (many of them closely resembling *Spirochaeta pallida*) similar to the spirochaete-like bodies seen in the inclusions found with the cells of guinea-pigs and earthworms. If these inclusions are burst the spirochaetes can be extruded.

All the forms here described have been seen in the chancres, glands, and sores of syphilitic patients, and also, but less frequently, as might have been expected, in the circulating blood. They have not been seen apart from syphilis, although a great many controls of blood and tissues have been examined on the jellies.

6. Homologies between the Human Parasites found and the Intracellular Parasites of Guinea-pigs and Earthworms.

The parasitic cell inclusion of the guinea-pig has been shown to develop into spirochaetes; the parasitic cell inclusion of the earthworms has been shown to develop into spirochaetes; the parasitic cell inclusion in human syphilis has been seen to develop into spirochaetes, and the presence of spirochaetes in syphilis is well known; but the human parasite has been found to develop into free, round, or pear-shaped bodies as well. It is true that free amoeboid forms were also found in the blood of guinea-pigs, and these have been mentioned by Hunter,⁶ but their parallel development in the guinea-pig had not been observed. When these pear-shaped bodies were seen in human syphilitic sores a search for the parallel development of the homologue in guinea-pigs was made, and these bodies were found by J. W. Cropper in the large mononuclear cells of the peritoneal cavity of infected guinea-pigs; and he has now observed their complete development exhibiting stages exactly similar to those of the development of the pear-shaped bodies observed in human syphilides. This discovery in the guinea-pig's

DESCRIPTION OF PLATE.

Fig. 1.—A small intracellular parasite commonly found in syphilitic chancres.

Fig. 2.—Division of the parasite chromatin; an inclusion found frequently in the mononuclear cells of the glands.

Fig. 3.—A cell from a chancre containing two parasites showing development. Note the copper colour of the parasites.

Fig. 4.—A mononuclear cell from a chancre containing three parasitic inclusions, two of which show the development of the spirochaetes, the third being an early stage of the phase shown in Fig. 6.

Fig. 5.—A large mononuclear cell found in the finger blood of a case of secondary (macular) syphilis. It shows the formation of the spirochaetes from a central chromatin mass within the included parasite.

Fig. 6.—A parasitic inclusion found in a cell of the blood taken from a syphilitic papule; it exhibits the subdivision of the chromatin of the parasite and the mode of the production of the free forms.

Figs. 7, 8, 9, 10.—The free forms—round and pear-shaped bodies—found commonly in the chancres, sores, and glands of syphilis; they are amoeboid, stain in a characteristic manner, and are found in the earliest thickening which initiates the Hunterian chancre, as well as in all the manifestations of secondary syphilis examined up to the present time; they contain granules and a central chromatin mass, which at first sight appears as a vacuole containing a central dot, but if watched on the jelly it will be seen to stain as in Fig. 8.

Figs. 1, 2, 3, 6, 8 very closely resemble the development of the female element of *Lymphocytozoon cobayae*, the homologous parasite of guinea-pigs. The rabbit parasites show similar phases.

Figs. 3, 4, 5 very closely resemble the development of the spirochaetes or male element of *Lymphocytozoon cobayae* and of *Spirochaeta lumbrici*, the homologous parasites of guinea-pigs and earthworms. The rabbit parasites show similar phases.

Figs. 11 to 15.—Peculiar bodies found in secondary and early tertiary syphilis. They occur frequently in the sores, non-suppurating glands, and have been seen in the blood of tertiary manifestations. Similar bodies to these are commonly seen in the blood of guinea-pigs infected with *Lymphocytozoon cobayae*, where they appear attached to mononuclear leucocytes, and in the seminal fluid of earthworms infected with *Spirochaeta lumbrici*, where they appear free as pictured here. In the guinea-pig these large granular bodies produce buds, each one of which, when separated, contains chromatin.

Fig. 16.—Two red blood corpuscles drawn for size comparison.

The figures are copied from specimens of living cells stained *in vitro* by the jelly method.

* The parasite has just been found by Cropper in the peritoneal cavity of newly born guinea-pigs.

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E. H. Ross del ad nat.

cells appears to give credit to the suggestion that the spirochaetes are really microgametes or male elements, and, if this should prove to be the case, the possibility of some of the round or pear-shaped bodies being macrogametes or female elements must be considered. This, however, could only be proved by observing the act of conjugation, and convincing oneself of the observation. But further evidence exists as to this parasite being a protozoon having a cycle of sporogony in the fact that in the deeper layers of the secondary syphilides peculiar cells are to be found. These vary in size from that of a large blood platelet to that of an enormous cell larger than the eosinophile. These cells are spherical, are completely studded with multitudes of small chromatin copper-coloured staining granules. They have been likened to mast cells, but the variation of their size, their bag-like shape, the numbers of granules and absence of nuclei serve to distinguish them. I have only seen these cells in syphilides.

7. The History of the Parasite of Syphilis.

Klebs¹ appears to have been the first to consider the parasite of syphilis in 1879; Losdorfer¹¹ and Döhle¹² also described parasites. But Stassano¹³ seems to have been the first to find cell inclusions in the manifestations of the disease. Then Siegel¹⁴ described his *Cytoryctes luis*. In that description he pictures certain flagellate bodies, the counterpart of which can be seen by the jelly method. These flagellated bodies very closely resemble the production of the spirochaete-like forms, but appear outside the cells. Schaudinn went to investigate Siegel's work and he discredited it. A few months later he produced his famous discovery¹⁵ of spirochaetes swimming free in syphilides, and these were named *Spirochaeta pallida*, and have been very generally accepted as the causative agents of syphilis. Schaudinn's work was confirmed and proved by Metchnikoff and Roux.¹⁶ It seems probable, however, that Stassano, Siegel, and Schaudinn can each claim to have discovered a phase of a parasite found in syphilides, though I understand that Siegel has lately modified some of his former views. It is important to mention that de Korte,¹⁷ while house-surgeon at the London Lock Hospital, described in 1906 free round bodies in the chancres of syphilides, and "a cyst measuring 12 μ , containing crystalline-looking threads, the whole staining very faintly." He called these protozoa, and classed them with the Ciliata; his illustrations are very poor. Recently I have heard that Mr. McDonagh, F.R.C.S., surgeon to the London Lock Hospital, has found cell inclusions in syphilis. Intracellular stages of various other spirochaetes have been described by many authorities—Leishman, Andrew Balfour, Fantham, Hindle, Blanc—but the relations between the various bodies described above and the *Spirochaeta pallida* were not hitherto demonstrated.

8. The Technic of the Jelly Method.

The jelly method, as invented by H. C. Ross,¹⁸ is as follows: 3 c.cm. of a 2 per cent. solution of agar in water, boiled and filtered; 1 c.cm. of Unna's polychrome methylene blue (Grübler), which has been previously diluted with two volumes of water—that is, 1 in 3; and 2 c.cm. of a solution containing 4.5 per cent. sodium citrate, 1.5 per cent. sodium chloride, and 0.225 per cent. atropine sulphate. This is boiled up together in a test tube and 0.3 c.cm. of a 5 per cent. solution of sodium bicarbonate ("alkali solution") added. Then a drop of this mixture when molten is poured on to a microscope slide and allowed to spread thereon, to cool, and to set. The chancre to be examined should be pricked with a large needle, and the blood squeezed from it so as to obtain the deeply situated cells. A drop of this blood—which, for convenience of conveyance and for the prevention of clotting, may be citrated with an equal quantity of 3 per cent. solution of sodium citrate and a 1 per cent solution of sodium chloride—is placed upon a cover-glass and inverted on to the set jelly. The blood spreads out between the cover-glass and the jelly in a thin film. In five minutes, when the cells have come to rest, the specimen can be examined under the microscope. The presence of the atropine in the jelly is optional, but it causes the cells to exhibit amoeboid movements, and this serves to differentiate the living cells from the dead ones and the stain enables the cell contents to be distin-

guished clearly. The granules of the leucocytes stain scarlet while the cells themselves are still motile; then as the nuclei stain—at first a pale blue, but later a deep ruby red—the motility ceases for the cells die. At length the stain fades from the cells, some of which may burst, for the cytoplasm liquefies and the cell becomes disorganized. It is not easy to make bad specimens by this method, and the pictures obtained are clear, each cell standing alone.

The parasites described generally stain after the granules of the leucocytes, and they appear as copper-coloured bodies either free—when they exhibit amoeboid movements—or included within the larger mononuclear cells. Each parasite shows the copper-coloured cytoplasm which contains a varying number of granules which stain deeply. In the centre of the parasite its "nucleus" appears, at first unstained, when it may be seen as a clear space showing a black dot in its interior. If the parasite is watched, however, the chromatin of the nucleus will be observed to stain suddenly a deep mauve, and then as the parasite dies the stain fades, leaving it a colourless sphere. This achromasia occurs to all the cells, and it is therefore essential to observe the specimen early; but the included parasite sometimes retains its stain after its host-cell has become achromatic. Occasionally in the external sores of syphilis the free parasites may be found engulfed by the polynuclear leucocytes. But in the non-suppurating glands and in the peripheral blood the larger mononuclear cells contain them, and it is in these cells that the development of the parasite may be observed; as stated before, however, the numbers of infected cells are much smaller in the peripheral blood than in the glands and sores.¹⁹

Some warnings are necessary. If the blood has been shed some time, or if there is too much alkali solution in the jelly, the cells may show diffusion vacuoles—small pink-staining dots, which may be mistaken for parasites—they possess no chromatin, however—indeed, they contain no structure, and they grow in size as the specimen stands until they burst. The parasite, on the other hand, has a clear cell wall, and possesses definite internal protozoal structure. If the patient from whom the blood is obtained has received treatment, such as an injection of "606," or of mercury, the free parasites are reduced in numbers, apparently within a few hours, whereas the included forms remain as before. This observation is based on but a few cases, and may require modification hereafter.

9. A Possible Means of Diagnosis.

It is too soon to be certain whether the presence of this parasite in the sores or blood can be regarded as a means of diagnosing syphilis. The evidence at present is strong. It has been found in syphilis only so far. The parasite develops into spirochaetes closely resembling the *Spirochaeta pallida*. The homologous parasite of guinea-pigs also develops similarly into spirochaetes. The homologous parasite of earthworms also develops similarly into spirochaetes. Guinea-pigs infected with *Lymphocytosoon cobayae* and the resulting spirochaetes exhibit gumma-like tumours, in which the parasites are found. The history of the finding of the parasites and its homologues points to its connexion with the causative agent of the disease. Still, I cannot say yet with absolute certainty that this protozoon is the cause of syphilis. The quickest way of proving this point will be the examination of as many cases as possible, and if others will co-operate in doing this it will soon settle the matter. By this means we shall obtain the real value of the finding of this parasite as an aid to diagnosis. I have found them in the early induration which initiates the formation of the Hunterian chancre; so far, from that time onwards, they have been found in all primary and secondary sores examined.

10. A Working Hypothesis.

Perhaps I may be permitted a suggestion. There seems justification for the suggestion that the guinea-pig or rabbit parasites might produce in man a mild affection which would modify the human disease of syphilis in the same way as the vaccination of cow-pox has modified small-pox

* Similar parasites to those found in syphilis have been found in the blood of rabbits. These rabbits exhibit chancres, buboes, ulcers of the genitals, mouth, anus, etc., and some have white patches of the liver like the infected guinea-pigs. The rabbit parasites, which are very numerous in the sores, possess phases indistinguishable from the human parasites.

in the individual and in the community. It must be remembered that the believed parasite of small-pox and vaccinia—the *Cytoryctes variolae* and *vaccinae* of Guarnieri—belongs to a family of cell inclusions (Chlamydozoa) somewhat similar to those I have described. Therefore the suggestion seems worth following up.

REFERENCES.

- ¹ Klebs, *Arch. f. exper. Path. u. Pharm.*, 1879, p. 161. ² By Dr. Ledingham. ³ Kurloff, Ehrlich's *Die Anaemie*. ⁴ Ehrlich (quoted by Ledingham), *Lancet*, June 16th, 1906. ⁵ Goldhorn, *New York Path. Soc. Proc.*, 1905. ⁶ Hunter, *Univ. Penn. Med. Bull.*, Philadelphia, vol. xxii, 1909. ⁷ Sir R. Ross, *Roy. Soc. Proc.*, B., vol. lxxxv, p. 71 (quoted). ⁸ Schilling, *Centrabl. f. Bakt.*, orig., vol. lviii, p. 4. ⁹ J. W. Cropper, *Roy. Soc. Proc.* (printing). ¹⁰ E. H. Ross, *Roy. Soc. Proc.*, B., vol. lxxxv, p. 67, 1912. ¹¹ Losdorfer, *Arch. f. Derm. u. Syph.*, 1872, and *Wien. klin. Woch.*, 1900. ¹² Döhle, *Centr. f. Bakt.*, 1892, and *Münch. med. Woch.*, 1897. ¹³ Stassano, *Acad. des Sciences. Paris*, 1901. ¹⁴ Siegel, *Abhandlungen d. h. preuss. Akad. d. Wissenschaften*, 1905, Ab. iii, S. 1-15, and *Münch. med. Woch.*, 1906, vol. i, p. 63. ¹⁵ Schaudinn, *Arbeiten aus d. Kaiser. Gesundheitsamt*, 1905, Bd. 22, p. 527-534; *Deut. med. Woch.*, May 4th, 1905, p. 711; and *BRITISH MEDICAL JOURNAL*, May 27th, 1905, p. 1161. ¹⁶ Metchnikoff and Roux, *Acad. de Méd. de Paris*, May 16th, 1905, and reported in the *BRITISH MEDICAL JOURNAL*, May 27th, 1905, p. 1178. ¹⁷ De Korte, *Practitioner*, June, 1906. ¹⁸ H. C. Ross, *Lancet*, January 16th, 1903, and *Roy. Soc. Proc.*, B., vol. lxxxii, p. 97, 1903.

THE "INFECTIVE GRANULE" AS THE INITIAL PHASE IN THE LIFE-HISTORY OF A HAEMOGREGARINE.

(Preliminary Note.)

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WHILE investigating the spirochaetosis of Sudanese fowls, Balfour of Khartoum¹ discovered that there occurred from time to time during the disease a period which he has called the after-phase, when the causal organism as such disappeared from the peripheral circulation, and when, coincident with this disappearance, the erythroblasts were found to contain certain curious intracorporeal bodies which he maintained to be a stage in the life-history of the spirochaete in question. Such an interpretation was much disputed at the time and still meets with opposition, but Balfour himself since his original communications has done much to throw light on the exact significance of these intracorporeal bodies.² Working with infected chickens in which a natural crisis has occurred, or in which an artificial crisis has been induced by salvarsan, he found by using dark-ground illumination that the spirochaetes break up into granules in the internal organs. These granules, to which have been given the name of "infective granules,"

are discharged by the still living spirochaete from one or other end of its periplastic sheath or cell membrane, and would appear to constitute a stage in the non-sexual life cycle of the parasite, for they seemingly enter the red cells, develop in them, undergo a process akin to schizogony, and regain the liquor sanguinis as tiny merozoites, which vary in number, and slightly also in size.

In working with the blood of the Gadidae, in one species of which—*Gadus pollachius*—I have found a spirochaete, I have from time to time met with corpuscular inclusions which I believe to be of the same significance as the bodies described by Balfour in chicken spirochaetosis. Similar appearances have been described, too, in the case of certain other non-pathogenic spirochaetes.³ Moreover, attention may be directed to the series of observations carried out by Sir William Leishman on the chromatin granules found to occur in the tissues of *Ornithodoros moubata* fed on blood containing *Spirochaeta duttoni*.⁴

In trypanosomiasis the so-called infective granule has been demonstrated by Major Fry,⁵ and observations with regard to this body in *Trypanosoma gambiense* have been published recently by Lieutenant Ranken, of the Sudan Sleeping Sickness Commission.⁶

Buchanan,⁷ too, in describing certain cultural phases of *Trypanosoma brucei*, ends with a rather significant remark:

A marked feature, perhaps worthy of note, was the constant presence of free chromatin granules in nearly all the smears from the second day onwards. These were apparently derived from the nuclei of disintegrating trypanosomes, and possibly represented a first phase in development.

While searching some blood films taken in 1909 from specimens of *Solea vulgaris* infected with *Haemogregarina simondi*,¹⁰ I noted that certain of the red corpuscles harboured in their cytoplasm two deeply staining granules having the appearance of chromatin. At the time I took these bodies to be of the nature of the centrosomic bodies described by Ross, Moore, and Walker.¹¹ Recently an opportunity has occurred of investigating a series of specimens of *Solea vulgaris* at Plymouth. In all of these fish *Haemogregarina simondi* could be demonstrated in the circulating blood or in the viscera. The parasite was examined in fresh blood, to which had been added N/2 sodium chloride containing a trace of certain dyes, in cultures of Nicolle's blood agar medium, and also in various fixed preparations. Most commonly the parasite occurs in the form of a colony of eight individuals all lying side by side inside the same red corpuscle, which is in consequence enormously enlarged (Fig. 1). The length of each individual varies from 18 to 22 μ . The anterior extremity is club-shaped, and often furnished with a small protocis. The posterior extremity is thinner and tapers

to a point, while the nucleus is situated somewhere in the middle third of the body. (Fig. 2.). While watching the escape of these parasites from infected corpuscles into the surrounding plasma I was struck by the fact that in many of the free haemogregarines one, two, or more bright refracting granules were found to emerge from the nucleus (Fig. 3) and to make their way through

the protoplasm of the parasite to one or other extremity, in the majority of cases to the posterior extremity. In this passage from the nucleus to the posterior extremity these granules frequently divide, so that two granules which have emerged from the nucleus become four when they reach the posterior extremity. (Fig. 4.) Further, these granules are shaken free from the living haemogregarine as it swims among