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.

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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

the corpuscles. The free granules, which are frequently to be met with in pairs, each individual being apparently connected to its fellow by an invisible thread, are much more highly refractile, and appear to exhibit more marked Brownian movement than do the free leucocyte granules and the other granules to be found in fresh blood preparations. On one occasion a pair of granules which were seen to escape from a free haemogregarine were found overnight to have taken up a position actually inside a neighbouring red blood corpuscle. Moreover, these two intracorporeal granules were found to have divided into four between 10.30 a.m. and 4 p.m. of the same day.

The extrusion of these granules from the nucleus of the parasite, their passage to the posterior extremity, and their liberation into the surrounding blood plasma, may be readily observed in films of fresh blood kept between slide and cover-slip, or in films made from blood kept in tubes of Nicolle's blood-agar medium. In the case of soles examined during May, 1911, granule formation occurred, as a rule, after the individual parasites had escaped from the host cell, whereas with soles examined in September, 1912, granule formation was in most cases marked while the parasites were still intracorporeal.

In living blood the granules readily take on a red staining with polychrome methylene blue, and they also take up pyronin with great avidity. With fixation followed by Romanowski stains they are large and well marked, while with iron haematoxylin in sublimate fixed wet smears they appear smaller.

Again, in specimens of blood showing a heavy infection with *Haemogregarina simondi* red corpuscles are frequently found containing in their cytoplasm granules which are indistinguishable from and probably identical with the granules extruded from the haemogregarines. These granules may be spherical or pyriform in outline, and would appear to be undergoing division inside the corpuscle, for they are frequently to be met with in twos and in fours (Fig. 5). Adjacent granules are often found to be connected by a delicate thread of chromatin. These intracorporeal bodies at first consist entirely of chromatin, but as they increase in size there appears round each a small clear zone of irregular outline (Fig. 6), which zone later becomes a definitely stained piece of protoplasm (Fig. 7). Phases showing this acquisition of protoplasm are met with occasionally in the blood stream, but more often in spleen and in liver smears. Also in spleen and in liver smears these bodies, composed of granules plus protoplasm, would appear to increase rapidly in size, so as to give rise to larger oval bodies which are distinctly haemogregarines (Fig. 8).

In the above short description I have not attempted to give the vertebrate life-history of *Haemogregarina simondi*, but have referred merely to a process of granule formation which presents a very striking resemblance to the formation of infective granules described in connexion with certain spirochaetes and trypanosomes.

A trypanosome of which I have preparations occurs in *Solea vulgaris*, but I have never met with this trypanosome in specimens of sole taken at Plymouth, and I have never found flagellates developing in any of my cultures.

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PROFESSOR EHRLICH has been elected a member of the Royal Bavarian Maximilian Order for Art and Science. This, we understand, is the highest distinction which Bavaria confers on scientists.

THE old "Freie Vereinigung der Chirurgen Berlins" (Free Union of Berlin Surgeons) has become extinct after an honourable and active life of a quarter of a century, and in its place a new society, called the Berlin Society of Surgery, has been formed. On November 11th Professor E. Sonnenburg, as President of the new society, delivered the opening address, in which he briefly sketched the history of the late union and indicated the lines upon which the new society would work.

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THE PARASITES RECENTLY FOUND IN
SYPHILIS.

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HAVING been informed by Mr. E. H. Ross that he had recently found a protozoal parasite always present in the lesions of secondary syphilis, and after he had shown me the details of the jelly method which he employs for its demonstration, I have repeated the technique and have examined some chancres and syphilitic blood. I have found the parasite which he described, and have traced some of its phases.

The jelly method: "coefficient jelly," consists of 2 per cent. agar, containing a sufficiency of saline solution to prevent cytotoxicity; 5 c.cm. of this is placed in a test-tube, and 0.4 c.cm. of Unna's polychrome methylene blue, and 0.4 c.cm. of a 5 per cent. solution of sodium bicarbonate in water is added; the total bulk of the mixture in the test-tube is then made up to 10 c.cm. This jelly is boiled, and when molten a drop is poured on to a microscope slide and allowed to spread into a thin film. When cool and set the blood or pus from a chancre is put on a cover-glass, and this is inverted on to the surface of the set jelly. The cells spread out between the cover-slip and jelly, and in a few minutes the leucocytes and parasites begin to stain, and can be examined.

The parasites appear as small, round, brown-coloured bodies lying free in the plasma. Each one contains some deeply staining granules and a vacuole (Fig. 1). Besides

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

these similar bodies are found enclosed within the mononuclear cells. In this situation they show up on the jelly as copper-coloured "inclusions," which on careful examination contain a stained structure (Figs. 2, 3). If the epithelial cells from the chancres are examined on the jelly, some of them also will be seen to contain these parasites, and some of these will be seen developed as pictured in Fig. 4. In these the central structure of the enclosed "inclusion" has developed into a bunch of spirochaetes; these spirochaetes diverge from a central stained mass like the spokes of a wheel. Some of the spirochaetes show the curly, wavy forms so typical of the *Spirochaeta pallida*.

The jelly method is so very simple that I have written this note with the view of its general adoption as a means of diagnosis in syphilis. One has only to see the phases as drawn in Fig. 4 to assure oneself that here is the development of the causative agent of the disease. The process of making the jellies requires but a few minutes, and each tubeful suffices to make many specimens. The time occupied in making each specimen is short. These parasites can also be seen in the peripheral blood of syphilitics, but here they are more scarce, and for diagnostic purposes I advise the examination of chancres and sores when several cases are to be examined in hospital practice or in venereal wards. If there is time blood examination is as satisfactory.

ON CERTAIN BODIES FOUND IN SYPHILITIC
LESIONS DEMONSTRATED BY THE
JELLY METHOD.

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MR. E. H. ROSS, of the John Howard McFadden Researches, the Lister Institute, first drew my attention, at the end of August, to his paper on the development of a leucocytozoon of guinea-pigs, which was published in the *Royal Society Proceedings*, B., vol. lxxxv, February, 1912. On September 4th he demonstrated to me the "jelly" method.