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 intracorpuscular 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 intracorpuscular.

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 intracorpuscular 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.

THE PARASITES RECENTLY FOUND IN SYPHILIS.

BY E. JENNINGS, D.P.H.CANTAB.,
LIEUTENANT-COLONEL, I.M.S.

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 cytolysis; 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

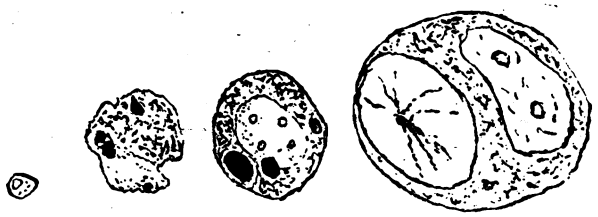


Fig. 1. Fig. 2. Fig. 3. Fig. 4.

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.

BY S. R. MOOLGAVKAR, M.R.C.S.,
HOUSE SURGEON, MALE LOCK HOSPITAL.

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.

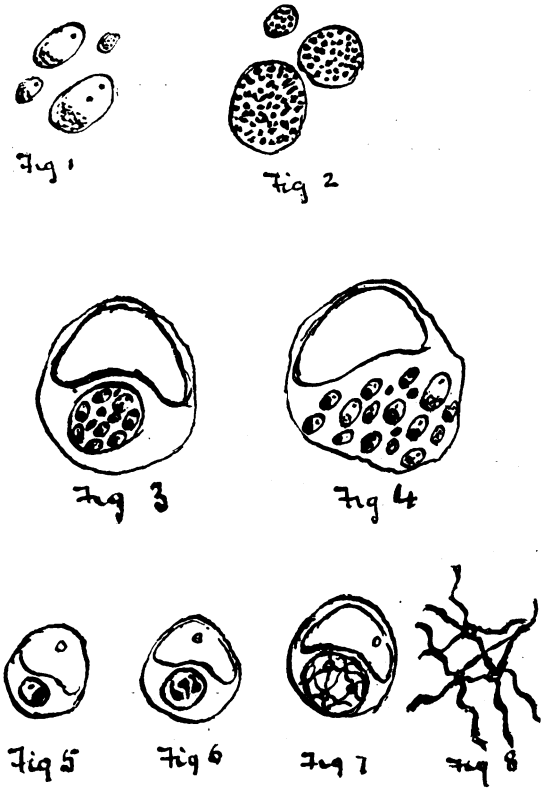
On September 9th he showed me some peculiar "bodies' which he had found by this method in syphilitic chancre scrapings. In consequence, I have followed this method, and here describe the bodies I have seen while working with the jellies myself.

I make the jellies as follows:

Agar, 1 gram; sodium chloride, 0.5 gram; polychrome methylene blue, 4 c.cm.; distilled water, 100 c.cm.; boil, filter, and store in test tubes. When required for use, the jelly is melted and a small quantity is poured on to a slide to cover an area of about a square inch and allowed to cool and set.

The syphilitic material to be examined is obtained from a hard chancre; a drop of the scraping is mixed with an equal quantity of a 3 per cent. solution of sodium citrate in water, and placed on a cover-slip, which is inverted on to the surface of the set jelly. Or a hard, "shotty" syphilitic gland is punctured with a Bayly needle and the gland squeezed; after a minute the needle is withdrawn, and the material contained in it is expelled into the citrate solution as before.

On the jelly the cells, leucocytes, epithelial cells, lymphocytes, stain a general blue; the "bodies" stain



red, and this serves to distinguish them readily. I have examined 25 chancres and 22 glands by this method, and have found the bodies in every syphilitic case. They may be described as (1) extracellular, (2) intracellular. The former consist of two forms: (*a*) Oval bodies 2 to 10 microns in diameter, containing a granular nucleus and one or two chromatin dots (Fig. 1); (*b*) bodies varying in size from 6 to 15 microns, in which I can find no definite nucleus, but they are completely filled with granules sometimes in violent movement (Fig. 2). (2) The intracellular bodies may be divided into two classes also: (*c*) A red-staining mass, embedded within the cytoplasm of large cells, containing several definite round or pear-shaped chromatin structures (Fig. 3). Sometimes this mass appears to have burst when the chromatin structures are found free inside the cell cytoplasm (Fig. 4); (*d*) a large mononuclear cell containing similar red-staining masses (Fig. 5), which show within their interior either chromatin rods (Fig. 6), or a skein or tangle (Fig. 7) of chromatin threads. Apparently these intracellular bodies burst out of the cells, for the threads can sometimes be seen near, but outside, the cells attached to a common chromatin ring (Fig. 8).

In addition to the above I have seen certain other forms about 10 microns in diameter, which stain very deeply and contain five to six chromatin granules. This form, which is free, frequently seems to divide into six daughters, each containing a chromatin granule. Occasionally in the glands, but frequently in the chancres, refractile bodies about a micron in size are found; each contains two chromatin dots. It is possible that this is but a phase, incompletely stained, of the free forms already described.

I am of the opinion that these "bodies" are parasites because of their peculiar staining reaction and because they develop while included within the cytoplasm of the cells of the chancres and glands. Up to the present I have only seen them in syphilitic subjects.

I beg to express my thanks to Mr. E. H. Ross for showing me the technique of the jelly method, and to the staff of the London Lock Hospital for permitting me to use the material obtained from their patients.

ON THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF TRYPANOSOMA RHODESIENSE IN GLOSSINA MORSITANS.

BY

ALLAN KINGHORN AND WARRINGTON YORKE.

(Sixth Interim Report of the Luangwa Sleeping Sickness Commission, British South Africa Company.)

In a previous communication[1] various observations were recorded which appeared to indicate decisively that the developmental cycle of *T. rhodesiense* in *Glossina morsitans* is influenced to a marked degree by the meteorological conditions to which the flies are subjected. It is unnecessary to repeat in detail the experiments upon which this conclusion was based, but the evidence may be briefly summarized.

1. At Nawalia, in the Luangwa Valley, where a relatively high mean temperature was experienced during the greater part of the year (75–84° F.), *T. rhodesiense* was readily transmitted at laboratory temperatures by both "bred" and "wild" *Glossina morsitans*.

2. At Ngoa, on the Congo-Zambesi watershed, where, during the cold season of the year, the mean temperature was much lower (59–63° F.), we were unable to transmit the parasite by means of *Glossina morsitans*.

3. The trypanosome was readily transmitted at Ngoa during the cold season by flies which were kept in an incubator at a mean temperature of about 80° F.

4. Corroborative evidence in support of this contention was afforded by an analysis of the valley transmission experiments, which showed that the largest proportion of infective flies was obtained in the hottest season of the year. Further, in the Luangwa Valley a larger percentage of "wild" *Glossina morsitans* were found to be infective during the hot season; and lastly, the percentage of flies naturally infected was very much greater in the valley than on the plateau.

During the past months further experiments were completed which indicated in the most decisive manner that the development of *T. rhodesiense* in *Glossina morsitans* is directly dependent on the temperature to which the flies are subjected.

A further attempt to transmit the parasite by *Glossina morsitans* at laboratory temperature was commenced on July 24th; 160 "wild" flies, previously shown to be non-infective by feeding on a healthy monkey, were utilized. They were fed for three days on a heavily infected guinea-pig, and afterwards for thirty-seven days on healthy monkeys, none of which became infected.

In all, therefore, a total of 680 flies were used in six attempts to transmit the human trypanosome by *Glossina morsitans* on the Congo-Zambesi watershed at mean temperatures ranging from 59 to 65° F., without obtaining a single infective fly, whereas at Nawalia, in the Luangwa Valley, of 330 flies used in similar experiments, 6, and probably 10, became infective at mean temperatures ranging from 75 to 84° F. Details of these experiments are given Tables I and II.

The incubator experiments described in the previous paper were repeated, but laboratory-bred flies were used instead of "wild" ones. Attention was drawn to the fact