markedly emaciated, the parietal peritoneum was not congested, and there was no effusion into the cavity. The liver and the spleen were normal; the gall-bladder was full. The intestines as a whole were congested and the small intestine showed dark purple patches. The contents from the duodenum to the rectum were fluid, of a dark green colour in the small intestine and of a yellowish green in the large. There was considerable gaseous distension. The lungs, the pleuræ, and the pericardium appeared normal. Cultures made from the blood were sterile, and those obtained from the intestinal contents and from deep scrapings of the mucous membrane failed to show the specific organism. Sections were made of the intestinal wall and they gave the following results. Duodenum: Some congestion and œdema of the mucous membrane, especially towards the lumen of the bowel. Jejunum: Congestion throughout the depth of the mucous membrane; swelling, granulation, and breaking up of the epithelium towards the lumen of the bowel; debris in the glands; some small round-celled deposit in the interglandular connective tissue. Ileum: Condition rather more marked than in the jejunum; a few interstitial small hæmorrhages in the mucous membrane. Large intestine: Congestion of the mucous membrane and a little round-celled deposit in the interglandular connective tissue. A bacillus morphologically resembling the bacillus F was seen in the ileum.

Experiments to Demonstrate the Presence or Absence of Extracellular and Intracellular Toxins.

In order to demonstrate the presence or absence of intracellular and extracellular toxins two sets of experiments were devised.

Intracellular toxin.—Inoculations of emulsions of agar cultures in 0.75 per cent. sodium chloride were given after sterilisation at 60° C. for 40 minutes. The sterility was tested by free inoculation of agar tubes and incubation at 35° C. The results were as follows. On April 6th a 48 hours old agar culture was emulsified in 10 cubic centimetres of normal saline solution. Of this five cubic centimetres were inoculated intraperitoneally into a guinea-pig. On the next day the animal died. The post-mortem examination disclosed that the middle part of the small intestine was greenish in colour and contained much gas and bile-stained fluid. Films from the peritoneal exudate, the blood, the liver, and the spleen were negative, and cultures from these also proved sterile. On April 9th a 24 hours old agar culture was again emulsified in 10 cubic centimetres of normal saline solution and one cubic centimetre of this was inoculated intraperitoneally into a guinea-pig. On the next day the animal was listless, but it thereafter recovered.

Extracellular toxin.—700 cubic centimetres of Martin's broth, contained in a Winchester quart bottle laid horizontally in the incubator to give as much air space as possible, were inoculated on the surface and incubated at 37° C. for 10 days. No surface growth occurred, but an abundant growth formed in the medium. The broth was filtered and tested for sterility. On March 31st five cubic centimetres were inoculated intraperitoneally into a guinea-pig. The result was entirely negative, the guinea-pig remaining without any symptom of illness. So far as these experiments go endotoxins are present and extracellular toxins are absent.

Virulence.—An attempt to gauge any variation in virulence due to passage through a series of guinea-pigs was made by inoculations of 24 hours old agar cultures carefully standardised on the Thoma-Zeiss stage by the method elsewhere described by us.¹ Eight inoculations were given and in each case where death resulted and in the first case where the animal was killed and the organism grown from the blood, the culture recovered was employed for the next experiment in the series. The results obtained indicate no appreciable rise or fall in the virulence. To insure death a dose of about 2,000,000,000 was required at the end as at the beginning of the experiments.

Immunity produced.—Incidentally some indication of the production of immunity was given by a guinea-pig in the above series. On June 4th it received 850,000,000 of a 24 hours old culture intraperitoneally. On the 5th it was listless but recovered. On the 9th it was re-inoculated with 2,400,000,000 of a 24 hours old culture from the same source as the former. The animal remained perfectly well; control guinea-pigs inoculated with the same dose died.

Conclusions.

The bacillus F was obtained from a case of epidemic diarrhoea. By its cultural reactions it is readily differentiated from the bacillus typhosus. The absence of indol formation separates it from Morgan's No. 1 bacillus. The presence of well-marked motility is sufficient to distinguish it from Morgan's bacilli Nos. 3 and 4 and from the dysentery bacilli. A reference to the agglutination reactions (Chart C) shows that there is a relationship between this organism and the bacillus typhosus and the paratyphoid bacillus B. The bacillus F is able to produce diarrhoea in animals and can be recovered from their stools. We believe therefore that it may be an agent in the production of epidemic diarrhoea.

During the summer of 1908 we have further investigated this disease and find that we have obtained a positive agglutination reaction varying from 1 in 25 to 1 in 100 in nearly half of the cases tested against the bacillus F. The specificity of this reaction we cannot yet affirm, but we hope later to publish a further report on the material obtained during the past summer.

A CASE OF PRIMARY SQUAMOUS-CELLED EPITHELIOMA OF THE EPIDIDYMIS.

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As we have been unable to find a similar case recorded we feel that the following is of such unusual interest that we consider ourselves justified in publishing it in full.

A police constable, aged 46 years, a patient of Mr. R. P. Marshall of Bermondsey, was admitted into Guy's Hospital on April 30th, 1907, for a "painful enlargement of the right testicle." During his 25 years' service in the police force he had suffered from influenza nine times, from quinsy once, and from pneumonia once. He said that his right testicle had always been a little larger than the left. There was no history of gonorrhoea or syphilis. On Christmas eve, 1906, he arrested a drunken man, who grasped and squeezed his right testicle rather severely, but no swelling resulted. A few weeks later he noticed a dragging pain in the testicle which was slightly swollen. The swelling gradually increased in size and became harder. On March 12th, 1907, he consulted Mr. Marshall, complaining of "a feeling of weight, a dragging pain in the right testicle, and of occasional vomiting." These symptoms became worse when the patient was standing. A diagnosis of chronic orchitis was made, but in spite of treatment by strapping, mercurial inunctions, and other local applications the swelling persisted and the man began to waste and to have more severe pain. Mr. Marshall noticed some softening at one spot and then favoured the diagnosis of tuberculous disease, and referred him to one of us at Guy's Hospital on April 18th.

At this time the right half of the scrotum was moderately enlarged and contained a swelling which was smooth, soft, and translucent in front and below but hard and fairly irregular posteriorly, where an enlarged epididymis could be recognised. The testicle itself was not enlarged. The scrotum was slightly adherent to the postero-inferior part of the swelling, which was very tender. The cord was slightly thickened at its lower part and a small, round, hard nodule was felt in it just within the external abdominal ring. At the time the nodule was thought to be in the vas deferens, which was, however, quite normal in size and consistency lower down. The vesiculæ seminales and the prostate were not enlarged or unduly tender. The urine and the act of micturition were normal and there were no signs of pulmonary phthisis. The patient was pale and in poor condition but not thin, his pulse was quick, and he seemed to be more ill than the local condition could well explain. No enlargement of the iliac or inguinal glands could be discovered by careful and repeated examinations. There was no growth upon any part of the skin of the body, in the

¹ Bio-chemical Journal, vol. iii., 1908.

mouth, or in the rectum and anus. There were no signs of any oesophageal disease.

A diagnosis of tuberculous disease of the epididymis and vas deferens with secondary hydrocele was made. It was thought that the disease was pursuing an unusually rapid course in this man, who looked considerably older than his years. Castration without delay was recommended. The unusual hardness of the epididymis gave rise to some misgiving and malignant disease was considered but excluded on account of the extreme rarity of primary growth of the epididymis.

Operation.—On May 2nd an incision three inches in length was made over the right inguinal canal, which was freely opened. The cremasteric fascia was incised and the infundibuliform fascia was exposed. Without opening the latter the spermatic cord was isolated as far as the internal abdominal ring, where it was transfixed and tied with catgut. It was then clamped and divided a little below the ligature. The cut surface was normal in appearance and the vas deferens was not enlarged. The testicle was then pressed upwards into the wound in the groin, and it was easily shelled out by blunt dissection. The separation was effected between the cremasteric and infundibuliform fasciæ, so that the testicle, epididymis, tunica vaginalis, and cord were removed *en masse* within their complete fibrous covering, which was slightly adherent to the lower and hinder part of the scrotum. Very little hæmorrhage resulted from the separation. The inguinal canal was firmly closed and narrowed, the lower margin of the wound in the external oblique being made to overlap the upper edge by means of a continuous catgut suture. The cutaneous wound was closed without drainage, but the empty right half of the scrotum was elevated and securely bandaged against the pubes so as to prevent oozing into it.

Primary union resulted and neither blood nor serum accumulated in the scrotum. On the evening of May 11th, after the patient had returned to bed, slight oedema of the right leg was noticed for the first time. When he was discharged on May 22nd he was fairly well but still pale, with a quick and poor pulse. He had no pain and his wound was quite sound.

Mr. Marshall gives the following excellent account of the later course and symptoms: "Soon after his discharge I noticed some oedema of both ankles; this increased and spread up the lower extremities until it became considerable and was only slightly lessened by raising the foot of the bed; later ascites appeared but never became considerable; also oedema of the buttocks and lumbar region. This oedema I put down as being due to secondary infection of the lumbar glands pressing on the inferior vena cava. The pain again became very severe (it was relieved by the operation), shooting from the groin up to the umbilicus and round to the back. The vomiting at times was quite beyond control. The scar and inguinal glands remained free from secondary infection, but there appeared to be some thickening in the neck of the scrotum. Bedsores formed and I procured a water bed for him. He gradually sank and became more wasted about the head, thorax, and upper extremities, while the abdomen and lower extremities remained water-logged; he died on June 23rd at the age of 46, his death being due, in my opinion, to exhaustion consequent upon the pain, vomiting, sleeplessness, &c., due to secondary deposits in the abdomen. The bedsores also probably contributed to the exhaustion. No post-mortem examination was made. There was no evidence of secondary deposits outside the abdomen."

Description of the parts removed at the operation.—The tunica vaginalis contained about one and a half ounces of clear, dark-yellow fluid. The testicle was normal in appearance and on section was found to be free from new growth. (This was confirmed by the microscopical examination.) The epididymis was considerably and uniformly enlarged. It was hard and presented several slight elevations on its surface. On section it was found to be entirely occupied by a firm new growth which was grey and almost translucent in appearance and traversed by fibrous trabeculæ. Scattered about in this new growth there were some small, yellow, caseous centres of necrosis. The growth had spread into the parietal layer of the tunica vaginalis on its mesial aspect, so that this structure was thickened and infiltrated for some considerable distance; it was, however, not invading the skin of the scrotum which was

quite normal in appearance and showed no signs of ulceration. The vas deferens was quite normal. In front of it, at the level of the external abdominal ring, there was a small swelling, which was, however, not attached to it. This swelling presented the same appearance as did the primary growth. Although it was not shown to be connected with the lymphatics or veins of the cord there can be little doubt that it had originated in the former.

Microscopic examination.—As the growth of the epididymis, the infiltrated portion of the tunica vaginalis, and the nodule in the spermatic cord presented the same appearances it will only be necessary to describe the former. This consists of a fibrous stroma surrounding large columns of cells which have the appearance of an ordinary squamous-celled carcinoma. (See figure.) The

Microscopic appearance of the growth of the epididymis. H, Horny cell-nests. G, Granules of keratohyalin.

cells are large with clear nuclei. There are numerous concentrically arranged cell-nests which stain a brownish colour by van Gieson, and a blue by Gram's method. This proves them to have undergone a horny change. Many of the cells in their neighbourhood are occupied by granules of keratohyalin. Every here and there a few very typical prickles can be demonstrated. The remains of the tubules of the epididymis are, in all the sections examined, denuded of their epithelium. The growth, however, appears to be spreading out from these tubules, the cells being more numerous and the columns which they form larger in their immediate vicinity. There are extensive areas of necrosis.

Conclusions.—The presence of prickles and of horny cell-nests proves this tumour to be a squamous-celled epithelioma. There are several possibilities as to its origin.

1. It may have been a secondary deposit of an epithelioma in some other situation. Against this view may be urged the fact that no such tumour was found, although a careful examination was made. Again, the clinical symptoms pointed to a primary affection of the epididymis. If there had been a concealed epithelioma—e.g., in the pharynx or oesophagus—it seems incredible that it should not have given rise to symptoms in some situation nearer home, since it must have been of long standing and pretty generally

disseminated to produce so large a swelling of the epididymis.

2. The epithelioma may have grown in an inclusion dermoid in the neighbourhood of the epididymis. There is no evidence to show that such was the case, since the epididymis was uniformly infiltrated and there were no signs of the presence of such a dermoid.

3. We think that neither of the above explanations is the correct one and consider the tumour to have originated primarily in the epithelium of the epididymis. This structure is, however, lined by columnar ciliated cells, and we should expect a primary epithelial new growth arising in it to be a columnar or possibly a spheroidal-celled carcinoma, but not a squamous epithelioma. Epitheliomata are, however, occasionally observed in other organs that are lined by columnar epithelium, such as the gall-bladder, the intestinal and respiratory tracts. These growths have been accounted for by supposing the presence of some displaced cells which normally develop into squamous epithelium; or by assuming that the columnar cells of the part change into squamous cells, a process which is known as metaplasia. Tumours are, no doubt, often explained by the assumption of displaced rudiments in organs in which it does not seem at all clear how such rudiments became included. The epididymis, however, is developed from the Wolffian duct, which in the embryo lies immediately under the external epiblast, from which, according to some observers, it is partly derived. Although this does not seem normally to be the case it is quite possible that the growing duct occasionally separates off some of the cells from the epiblast. These epiblastic cells become incorporated with the duct and remain in an inactive condition until from some cause, such as an injury to the scrotum, and the inflammation thereby set up, they proliferate and assume malignant characters. They would necessarily give rise to an epiblastic carcinoma—i.e., a squamous epithelioma.

Metaplasia is in some organs, in which there is no possibility of such rudiments having been included, the only hypothesis. In the epididymis, however, which is developed so close to the epiblast, we think that the assumption of included epiblastic cells is the true explanation.

THE PARASITE OF KALA AZAR AND ALLIED ORGANISMS.¹

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SHORTLY after my return to England I was asked by our secretary to read a paper on kala azar before the society, and on considering what particular aspect of the disease I was most competent to deal with I decided to limit myself to the parasite, and to-night I propose giving you some account of my observations on this organism and its allies. I have no doubt you are all well acquainted with the parasite the discovery of which has opened up an entirely new field of research of great practical importance. Although we now know a great deal about it and its two allies, the parasites of infantile splenomegaly and Oriental sore, there is considerable doubt as to their exact systematic position. Laveran and Mesnil, I believe, still regard the parasite of kala azar as belonging to the genus *Piroplasma*, while you will remember that Ross placed it in an entirely new genus, *Leishmania*. The view that this parasite is a hæmocytozoon can no longer be entertained, and Christophers's recent studies on the extra-corporeal life-cycle of *Piroplasma canis* in the tick have shown that it does not pass through a flagellate stage similar to that of the parasite of kala azar. I am aware that certain observers claim to have discovered flagellate stages in some *Piroplasmata*; their observations, however, are far from convincing. I have recently seen some of these so-called *Piroplasma* flagellates and have no hesitation in saying that they do not bear any resemblance to the characteristic flagellates of the parasite of kala azar. It should also be noted that these flagellate stages of *Piroplasmata* have so far only been seen in test-tubes or in the blood of the

vertebrate host, whereas they ought to occur naturally in ticks or other invertebrates. There is, however, no doubt that the parasite of kala azar and its two allies as seen in man are but stages in the life-histories of flagellates, and the important question to answer is, Do they belong to any known group of the parasitic flagellata? As our knowledge of the life-cycles of these organisms is exceedingly limited I think it somewhat premature to say they belong to an entirely new genus, and I hope to show you that they do belong to a group which has long been recognised as occurring in the alimentary tracts of insects. From a study of the flagellates of the parasite of kala azar in the test-tube as well as in the bed bug *Cimex rotundatus* I have no hesitation in saying it is not a trypanosome, for like all other observers who have studied this stage I have never seen anything similar to the well-known vertebrate trypanosome.

The most important suggestion as to the nature of the parasite of kala azar was undoubtedly made by Rogers who, on comparing it with *Herpetomonas muscæ domesticæ* as figured by Prowazek, recognised the close similarity in the build of the two flagellates. Unfortunately, Prowazek erroneously described the adult flagellate of *Herpetomonas muscæ domesticæ* as possessing two flagella closely united together, and in addition its life-cycle according to his account is exceedingly complicated. For more than a year I have made an exhaustive study of this flagellate, the type species of the genus *Herpetomonas*. In Madras about 100 per cent. of flies caught in the bazaar meat shops are infected with it and as a result of my observations on the living parasite as well as in stained preparations I am unable to accept Prowazek's view of its flagellar apparatus, for I find the adult flagellate has a single flagellum. I can only assume that Prowazek happened to examine flies in which the majority of the adult flagellates were undergoing the early stages of simple longitudinal division. I have frequently seen this in Madras. I also find that the life-cycle of the flagellate of the house fly is very simple and is similar to that of the parasite of kala azar as far as we know it at present.

You will remember that Prowazek began his description of *Herpetomonas muscæ domesticæ* at its adult flagellate stage and entirely omitted the stage preceding it, thus giving anyone who read his paper an entirely erroneous idea of its life-cycle. As you will see later the earlier stages in the development of the parasite are of great importance as it is this stage when it is so like the parasite of kala azar. In order therefore to clear up this difficulty I will describe in some detail the life-history of *Herpetomonas muscæ domesticæ*, the complete account of which I shall reserve for another occasion. After having studied a large number of these parasites I have found it convenient to divide their life-cycles into three stages, *pre-flagellate*, *flagellate*, and *post-flagellate*, and it should be clearly understood there is no hard and fast line dividing one stage from the other, they merge into each other. You will, however, see that by studying these flagellates along these lines it is easy to work out their complete life-histories, and above all to find out how they pass from one host to another. In the case of *Herpetomonas muscæ domesticæ* in Madras the flagellate and post-flagellate stages are readily found, but owing to the fact that a large number of flies have to be examined before the pre-flagellate stages can be studied, this important portion of the life-cycle of the parasite is very apt to be missed.

The pre-flagellate (Fig. 1, Figs. 1 to 3A) stage is usually found in the midgut, the parasites lying in masses within the peritrichal membrane; they are round or slightly oval bodies measuring on an average about 5.5μ in breadth and contain a nucleus, blepharoplast, and a number of granules. They multiply by simple longitudinal fission or by multiple segmentation (Figs. 2, 2A, 3, 3A), so that very soon a large number of these bodies are formed. The flagellate stage is characterised by the formation of the flagellum, a single stout filament which projects freely from a point in close proximity to the blepharoplast (Fig. 4), the resulting flagellate elongates and later divides by simple longitudinal division (Figs. 5, 6, and 7). The nucleus of the adult flagellate lies about the centre; the blepharoplast, a large rod-shaped body, is nearly always situated a short distance from the anterior end; the flagellum is a single filament which projects freely almost at once from the rounded anterior end. The early stage of the process of longitudinal fission begins by a splitting of the root of the flagellum and this, as well as further stages of the same change, led

¹ A paper read before the Society of Tropical Medicine and Hygiene on Jan. 15th, 1909.