jaws and much general muscular rigidity, and the patient was not fully convalescent till September 28th, two months and four days from the date of the accident.

During the course of the illness the most prominent symptoms were rigidity of the muscles of the jaws, back, abdomen, and upper part of the thighs, accompanied by frequent clonic spasms at irregular intervals; the muscles of the upper extremities were but little affected. Swallowing was accomplished with great difficulty, but fortunately the act of deglutition never failed completely. Tenacious mucus accumulated in the pharynx, causing much trouble and distress. The bowels were constipated; urine passed without difficulty. Temperature varied between 101° and 102° , and on one occasion reached 103° . Pulse became very rapid, sometimes almost uncountable, and even when convalescence was fully established remained well over 80.

From the third day and for seventeen subsequent days 20 c.cm. antitetanus serum were injected every twelve hours, till on August 20th the patient was able to open his mouth a little way, the spasms being also less frequent, so the dose of serum was reduced to 10 c.cm. twice in twenty-four hours; the improvement being maintained, the dose of serum was reduced still further, to 10 c.cm. once in twenty-four hours for the next five days; on the twenty-seventh day the serum was omitted altogether for twenty-four hours; the rigidity of the muscles was much less marked, and there was only one spasmodic seizure, which proved to be last, on the twenty-eighth day; as a precaution 10 c.cm. of serum was administered for the last time. Though the patient had the large number of 83 injections under the skin of the abdomen and flank, not one caused more than the temporary discomfort due to the insertion of the needle. Ten days from the commencement of the serum treatment the patient was covered with a profuse scarlatinoid rash, which caused him a good deal of discomfort, and which was followed by general desquamation. The bromide and chloral mixture was given as occasion required. As it was impossible to clear out the tenacious mucus that accumulated in the pharynx the patient was frequently turned on to his side with his shoulders projecting over the edge of the bed, and his head hanging down, supported sometimes for an hour or more by his mother or the nurse; this position was assumed by the patient of his own accord, as in this way the mucus escaped out of his mouth and cleared his throat.

A turpentine enema was administered every third day, producing evacuation of the bowels. The wound caused no anxiety; it was treated with boric acid fomentations, and eventually granulated up.

The diet consisted of two to four pints of milk every twenty-four hours, the patient preferring this to any other form of liquid food.

In reviewing the case I think I ought to have injected a larger dose of antitetanus serum on the first occasion, but my want of knowledge of the treatment made "I dare not, wait upon I would!" If in the future I should have another case of acute tetanus I should begin by injecting at least 20 c.cm., and preferably by the intravenous method.

III.—By C. R. HOWARD, M.B., B.C. CANTAB., WOOLSTON, HANTS.

ON November 26th, 1906, J. E. grazed his hand when working on a troopship. Two days later he began to complain of aching and stiffness in the jaws, and on the fourth day had marked risus sardonicus. On the fifth day after the injury spasms appeared and treatment was commenced; it consisted in keeping the patient quiet by means of silence, darkness, and drugs, feeding him, excising the wound, and injecting antitetanic serum. Of the latter 110 c.cm. in all were administered on the following dates—December 1st, 5th, 9th, 11th, 12th. The injections were given subcutaneously in the back, and seem to have exercised a most beneficial influence, but they apparently caused an intensely irritative erythematous rash to develop.

The rigidity of the body decreased about December 9th, but the spasms continued till December 11th. They were severe on several occasions, and had to be controlled by chloroform, chloral and bromide, or morphine, of which during December 3rd and 4th about gr. iij were given.

The bowels were at first very confined, and croton oil had to be given on two occasions, but subsequently they were regulated by means of magnesium sulphate 3 iij every four hours. The motions were most offensive, greatly increasing the labour and discomfort of nursing. This was overcome by 5-gr. doses of salol given occasionally. During the first day or two of the illness much anxiety was caused by persistent vomiting, but a mixture containing bismuth, compound tincture of cardamoms, chloroform, morphine, and hydrocyanic acid, relieved this

symptom, which subsided as soon as the bowels were well opened. After this the patient took nourishment very well, the total amount being sometimes as much as eight pints of various liquids, such as milk tea, milk coffee, milk cocoa, Benger's food, broths, etc., in twenty-four hours.

The patient began to sit up during the first week in January, and since then has made steady progress.

The notable points in this case appear to be:

1. The short incubation period, combined with recovery.
2. The beneficial effect of the antitoxic serum given subcutaneously. This was obtained through Messrs. Burroughs and Wellcome.

THE STAINING OF ANIMAL PARASITES.

By I. WALKER HALL, M.D.,

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WHILE many of the higher parasites do not require any special staining in order to demonstrate their anatomical structure, their ova and embryos may sometimes escape detection when unstained, especially if they are present in small numbers. This is particularly the case when it is necessary to examine secretions such as the urine or faeces, or the contents of parasitic cysts. Staining is also an advantage in demonstrating embryos, ova, or sections of the entire animal, either by the aid of the microscope or by the lantern microscope.

These facts were brought home to the writer while endeavouring to project preparations of filaria on to a screen, and while examining specimens of faeces in which the ova were difficult to find.

Under such circumstances the following device was employed, and as in its entirety the procedure does not appear in the available literature, it is here described in the hope that it may obtain further application.

METHOD.

1. Prepare a film of the blood, faeces, or sediment of the secretion. Fix by heat, or, less preferably, by formalin vapour.
2. Treat the film with the following solution, $\frac{1}{2}$ to 2 minutes: Watery methylene blue, 1 per cent., 100 c.cm.; glacial acetic acid, 5 c.cm. (Neisser's methylene blue solution may be equally well used if at hand. Methyl violet, Nile blue, etc., are not so good.)
3. Wash in water.
4. Cover the film with saturated alcoholic eosin solution. Allow to stain for 5 to 10 minutes, or (following Muir's excellent method described in the *Journal of Pathology*, 1906) heat over the flame, blowing out the alcohol each time it catches fire, until the film is almost dry.
5. Wash in water.
6. Fix in potash alum solution, $\frac{1}{2}$ to 2 minutes.
7. Decolourize in 90 per cent. alcohol until a uniformly pink colour obtains.
8. Wash in water; allow to dry; mount in Canada balsam.

It is the treatment with acetic acid which affects the outer coverings of the ova, embryos, and parasites, and allows the stain to permeate the entire structure.

APPLICATION.

Faeces—Spread a film of the faeces on a large slide. A quarter photographic plate from which the film has been removed may be utilized. The acid solution decolourizes the faecal material and softens the membranes of the ova. After stage 6 the decolourization is continued until the ground stain is a light pink. Using a Zeiss A lens the ova are then seen as small deep-red spherules or ovoids. Their detailed structure is well defined under the high power.

Sediments from Secretions and Cyst Contents.—By these means the protein constituents are stained a uniform light eosin shade. Hooklets and ova are stained red, and stand out sharply against the pink background.

Filaria.—For the demonstration of these and other organisms infesting the blood stream, Leishman's method gives results far superior to those obtained by using Jenner's dye, eosin and methylene blue, haematoxylin and eosin, iron alum haematoxylin, fuchsin, or resorcin-fuchsin. It stains the sheath a delicate pink, the granular material a deep blue, and both "spots," as well as the muscular cutaneous layers, are well defined. An advantage possessed by this acid-eosin method over that of Leishman is that the sheath stands out sharply in light red, the body is a bright, deep red, and the granular material purple, and a better lantern picture can be obtained. With Leishman's stain the red corpuscles are terra cotta and the white corpuscles present the usual colouring of the granules. With this staining method the red corpuscles are only outlined, but the coarsely eosinophilous granules

stain deeply and are easy to count. The total result is very effective.

Adult Parasites.—When segments are stained in this way the internal structures, particularly the reproductive organs and the ova, are well brought out. It is best to allow the parasites or the segments to remain in the acid solution for about one hour, and in the eosin solution over a night. After thorough drying with absorbent paper they are cleared in clove oil or xylol for twenty-four hours and then mounted in balsam.

Spores.—My colleague, Dr. J. A. Nixon, working with certain fungi of the skin and hair, informs me that he finds this device gives very satisfactory results with those spores which are only stained with difficulty by the methods usually employed for this purpose.

The Milroy Lectures

ON

KALA-AZAR,

DELIVERED BEFORE THE ROYAL COLLEGE OF PHYSICIANS OF LONDON.

By LEONARD ROGERS, M.D., F.R.C.P., I.M.S.,
PROFESSOR OF PATHOLOGY, CALCUTTA.

LECTURE III.

THE LIFE-HISTORY OF THE PARASITE, MODE OF INFECTION, AND PROPHYLAXIS.

[ABSTRACT.]

I HAVE now dealt with kala-azar both collectively as an epidemic and individually as a disease, but have still to discuss the evidence I obtained in Assam regarding its infectiousness before describing the life-history of the parasite which produces it.

When I went to Assam in 1896 there was much difference of opinion as to whether kala-azar was infectious or not, the majority holding that as it was certainly malarial it therefore could not be infectious, while a few contended that it was certainly a communicable disease, and therefore it could not be simply malarial in nature, but no one had found time to inquire into the exact way in which it spread. In order to settle this important point I travelled over 150 miles on foot from village to village, and obtained the histories of the introduction of the disease into them from the head men, who return all the deaths, and who were always able to give me detailed information on the subject. Very similar stories were constantly related to the effect that some one suffering from the disease had come to live in the village, usually with relatives, and then others, who resided in the same house, were shortly afterwards attacked, and on the survivors of the first infected household being taken into other families they in turn suffered, while after a year or two the disease became widespread, and frequently from one to two thirds of the people eventually died of it before it began to decrease. Wherever the infection had spread a similar kind of history was obtained, while whenever a village had escaped, although surrounded by infected ones, it was found that for some reason or other they had no communication with each other, even if only a few hundred yards apart. Thus a small village of Brahmans, who had no intercourse with the infected low caste people around them, escaped, although all the surrounding villages had been attacked for several years. Again, one part of a village, which was separated from the rest by only a few small rice-fields, remained free for years owing to their head man not allowing them to have any intercourse, even with their relations in the infected portion. On the other hand, on visiting a village which was reported to be infected, although all those around it were free, I found that it belonged to people who had recently moved there from a badly-infected part of the district, bringing the disease with them. The extreme steps which the people themselves sometimes took to prevent the spread of the disease is also strong evidence of its infectiousness in their eyes. For example, a girl, who had married a man in a healthy village, but soon after was found to be suffering from kala-azar, was turned out, while her own father, in a neighbouring uninfected village, was prevented from taking her into his house, so she was living in a hut erected in the open rice-fields at the time of my visit. The same plan of

isolating cases of the disease was adopted by the Garos, who are reported to have gone so far as to render the patients comatose with drink and then burn them in their huts. All the evidence goes to show that the infection is, as a rule, obtained by sleeping in a house with infected persons for a longer or shorter time, in some cases only for a day or two. Moreover, the plan of moving the village to a new site appeared to have a distinctly good effect in lessening the disease when occasionally resorted to by the people themselves, so that I thought the infection might possibly pass through the soil. It was on the knowledge of the infection being largely a house one that I based the prophylactic measures which proved so successful long before the parasite of kala-azar was discovered, but now that we possess much more definite information regarding the true pathology of the disease it will be best to describe the life-history of the protozoal organism of kala-azar before taking up the important practical question of prophylaxis, as the latter will thus be rendered more definite.

THE PARASITE OF KALA-AZAR AND ITS DISTRIBUTION IN THE HUMAN BODY.

The parasite of kala-azar was independently found by more than one observer, but to W. B. Leishman, R.A.M.C., belongs the credit of having had the courage to first describe it, two and a half years after he had seen it, as probably a degenerate stage of a human trypanosome, not very long after that organism had been found in a fever in Africa by the late Dr. Dutton, while Rose Bradford and Plimmer had previously described a form of the *Trypanosoma brucei* similar to the bodies Leishman found. As soon as Leishman's paper reached India, C. Donovan, I.M.S., recorded that he had independently observed the same bodies in the spleens of patients dying of prolonged fever in Madras one month before Leishman's note was published, and as he was also able to find them in fresh spleen blood obtained by puncture of the organ during life, he was able to disprove Leishman's suggestion that they were degenerate trypanosomes, for the flagellate form of the organism has never been found in the human system. Laveran and Mesnil, after examining specimens sent by Donovan, concluded that the organism was a piroplasma, while J. H. Wright of Boston, at the end of 1903, described similar bodies to those of Leishman and Donovan in a form of chronic ulceration met with in the Punjab known as Delhi boil, in which D. D. Cunningham had, as early as 1885, described the aggregations of these peculiar bodies. As kala-azar is unknown in the Punjab there is good reason to believe that the parasite of Delhi boil is quite a distinct species from that of the Assam fever.

In 1896 I had stained smears of kala-azar spleens with aniline dyes without being able to distinguish these bodies, which are not well shown by them, while Romanowsky's stain and its numerous modifications had not then come into common use, and I mainly used fresh unstained blood films, as then advised by Sir Patrick Manson and others, for searching for malarial or other protozoal parasites. The papers just referred to appeared while I was on leave in England, and I quickly realized, as was also suggested by Donovan, Ross, and others, that if many of the cases previously known as "malarial cachexia" were really caused by the new parasites, then kala-azar of Assam must be also produced by it, for I had shown in my report that the Assam outbreak was but an epidemic form of the so-called "malarial cachexia." On returning to India at the end of 1903 I obtained some slides of spleen blood of kala-azar patients from my friend, Dr. Dodds Price, and readily found the new parasites in them, as well as in several cases of chronic spleen fever in the Dinajpur district, which were evidently the sporadic form of the same disease. At about the same time Dr. Bentley independently found the same parasites in kala-azar cases, and published his observations shortly before my own appeared in print. Other cases in Dinajpur with large spleens following chronic fever showed only malarial parasites in their spleen blood; and I found it impossible at a single examination to differentiate between the two forms clinically, although subsequently able to do so by the blood changes, as described in my last lecture. It is not, therefore, surprising that kala-azar has been so long confounded with chronic forms of malarial fever; and the discovery of the new parasite has done more towards clearing up the fevers of Bengal.