all our efforts to diminution of suffering until such time as the patient succumbs to sheer exhaustion, or dies from some concomitant complaint. It has been my lot to have such a case under almost daily observation for two and a-half years—from the first diagnosis of the disease to its fatal termination—and the course of the case under the treatment adopted seems to me worth recording, and the treatment itself worth remarking upon.

The problem I wish to draw attention to is this: Given a case of cancer where surgical treatment is impossible, what treatment offers the best means of making the patient's life bearable as long as it lasts?

The case I refer to was as follows:

Mrs. M., widow, aged 71, consulted me in May, 1898, for symptoms pointing to commencing cancer of the cervix.

HISTORY.

As usual, a slight hæmorrhage was the first thing complained of. Shortly afterwards an offensive discharge set in. A microscopic examination of a scraping from the cervix revealed the characteristic cell growth. The patient went to London with a view to being operated upon, and was seen by several surgeons in consultation. After careful consideration, however, it was decided that on account of the age (and other reasons) complete removal of the disease by surgical interference was inadvisable, and she returned home to the country. While in London—a period of some two months—pain began to require relief, and when she returned to my care was becoming severe. The pain increased rapidly in severity, and for a time the patient got much worse; then, under the treatment to be described immediately, she improved much in general health, and lived in comparative ease until October, 1900, when she had fainting fits on several successive days, and died from exhaustion.

TREATMENT.

(A) *Hæmorrhage*.—This was always controlled by ergot internally, and on the few occasions when severe flooding occurred, by subcutaneous injection of ergotin.

(B) *Offensive Discharge*.—In the earlier stages a solution of sulphate of zinc was used, but later I found nothing so satisfactory as a carbolic douche (1 to 40).

(C) *Pain*.—This soon became the great object of treatment, and about six months after the first diagnosis—a period which had been looked upon as the probable duration of life in this case—the pain was constant and intense. It had been treated till then with liq. morph. hydroch. alternating with chloral hydrate and morphine suppositories. But these began to lose their effect, and I was continually called up at night to give hypodermic injections of morphine to procure any sleep at all. The relief thus obtained was only evanescent, and it was evident the patient was rapidly being worn out by the intense suffering. I then remembered a remark of an old teacher of mine at a clinic on a similar case, when he wound up his words on treatment thus: "Then, gentlemen, if everything else fails the best thing you can do is to turn your patient into an unconscious opium eater." At this time (December, 1898) she was taking from 3 to 5 grains of opium a day in one form or another. I then decided to push the opium by internal administration alone, and finding that the tincture of opium had a more lasting effect, dose for dose, than the liquor morphinæ, I discarded the latter and used the tincture entirely. I gave 15m every four hours, or about 6 grains of opium in twenty-four hours; increased this to a dose every three hours in a few days, or 8 grs. a day, and found in a fortnight that the patient got entire freedom from pain for six or eight hours at a stretch, during which refreshing sleep was obtained. She soon began to show a marked improvement in her general condition, took food well, and instead of dying as was expected a few weeks before, began to get out in her carriage a little. The dose had to be constantly increased, however, to secure this freedom from pain, and not to make the description too tedious I may say at once that in six months' time (June, 1899) she was taking 3 ozs. of tincture of opium daily, or about 90 grs. of opium. A month later it had increased to 4 ozs., and on my going away for my summer holiday at that time my *locum tenens* flatly refused to make up the prescription until he had seen me do so and administer it. While away from home I met a well-known professor of surgery, and being rather anxious about the treatment I was pursuing, I told him of the case and asked his advice. He asked me if the patient slept well, took her food well, had regular action of the bowels, whether she had any head symptoms, and as to her general health. On my answering all these queries satisfactorily, he turned and said, "Then what on earth more do you want? Give her a pint if necessary." I took his advice, if not quite literally, and continued to increase the dose as tolerance became established, the only difference being that I added mv of tinct. belladonna night and morning, which I found assisted the action of the opium. By August, 1900, she was taking 6 ozs. of tinct. opii daily, or about 198 grs. of opium a day. Three months later this had risen to 8 ozs. or 264 grs. per day, and on some days, when the pain did threaten to become severe, she took over 12 ozs., or nearly 400 grs. She went on with this dose, one day a little more, the next a little less, till October, 1900, when she collapsed somewhat suddenly, and after a week of extreme prostration died.

The patient had thus lived two years longer than was at first thought probable, and I have no hesitation in saying that the last 18 months of life had been incomparably more comfortable than were the first 12 after the disease showed itself. Of course, I do not mean to say she had no pain at all; she had severe pain at times, but it was bearable, and never so distressing as it had been in the early stages. Throughout the whole of the last two years there was no difficulty with the bowels, no head symptoms, and a plain

milk diet was well taken. Towards the end the growth had extended to the bladder, and finally to the rectum also.

Looking back on the case, the firm impression left on my mind is that in no other way could the patient have lived as comfortably as she did or so long. She never knew that she took opium, and would occasionally ask me if the pain came on if I would give her a little morphine. Under similar circumstances I do not know that one can do better than follow the maxim quoted above, and "turn the patient into an unconscious opium eater."

The only drawback I see to this instrument is its great expense for the poorer class; and I may say that the druggist's bill in this case for tinctura opii alone was over £20. This, however, has nothing to do with the purely medical aspect of the treatment I have described.

THE APPLICATION OF ROMANOWSKY'S STAIN IN MALARIA.

[With Coloured Plate.]

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THE following note on the application of Romanowsky's method of staining for the demonstration of the presence of malarial parasites in the blood and, in particular, for the differential diagnosis of the tertian parasite, has been written in the hope that the simplified technique described below may render its great advantages more generally available to those who are brought into every-day contact with the disease.

The recent arrival at Netley of several large batches of invalids from India has given me the opportunity of using this stain in many cases of malaria, and the results obtained by it have proved strikingly superior to those in which other and better-known methods were followed, and I am convinced that those who may be led to give it a trial will appreciate the increased facilities it affords for the investigation of many important points in connection with the diagnosis, prognosis and treatment of malarial fevers. The method which has been followed in most of its essentials is that described with great care and detail by Dr. Georg Maurer¹ as the result of his work in Sumatra.

The chief advantages which the staining method described below holds over others are the following:

1. The specific action of the stain upon all red blood corpuscles infected by the tertian parasite.
2. The greater certainty of the detection of very young intracorpuscular forms of all varieties of the malarial parasite.
3. The facility with which the occurrence of a "mixed" infection may be detected, that is, the presence in the system simultaneously of more than one variety of the parasite.
4. After a little practice, the ease of its application and the certainty of the results obtained.

With regard to the first of these points—the specific action upon the red blood corpuscles infected by tertian parasites—the appearance in the infected cell, when stained by this method, of bright red dots or points was first pointed out by Schüffner.² A red cell infected by one of these tertian parasites forms one of the most striking of microscopical objects, as may be judged by a reference to Figs. 5-23 in the plate, which were all drawn from Nature from films prepared in the wards of this hospital and stained by Romanowsky's method.

The origin and nature of these dots are not by any means clear. The fact that their staining reaction is identical with that of the nuclear chromatin of the parasites might point to their being of similar structure; but, more probably, they are the result of trophic changes in the substance of the red cell brought about by the invading parasite, and are not directly connected with the parasite itself. Whatever their nature, they appear as fine more or less rounded granules scattered irregularly throughout the whole of the red cell, and, in the case of the more fully developed parasites, both over and underlying the parasite itself (see Figs. 5-23). They stain a deep ruby red colour, and are so numerous as to recall

the fine eosinophile granules of the polynuclear leucocytes. Maurer lays stress on the fact that, from the time they make their first appearance, they increase in size only and not in number; but, in the forms sketched in Figs. 5-12—which show a very young stage of the tertian parasite, and were all taken from the same case—it will be noticed that the dots are both larger in size and fewer in number than those of another case (Figs. 13-23) in which the parasites had reached a more mature stage. In fresh unstained films and in those stained by other methods these dots were quite invisible.

The cases of malarial fever in soldiers which come under treatment in Netley are naturally of a very chronic type, and their differential diagnosis is rendered difficult by reason of previous prolonged treatment by quinine and other antiperiodics and, as I shall have occasion to refer to again, by the occurrence of mixed infections. It is, therefore, but rarely that a case can be classified accurately as quartan, tertian, or pernicious by means of the clinical signs alone, and in only one instance—in the case of a recrudescence of malarial fever in a patient invalided for another cause—was it found possible to infer the microscopical from the clinical signs. The appearance, however, of Schüffner's dots is so unique and, as far as my experience extends, so constant that I have little doubt that those who have the opportunity of applying this stain in cases of fresh infection in a malarious climate will find, as did Maurer under similar circumstances, that they are invariably present and occur only in red blood corpuscles infected by the tertian parasite.

Secondly, the advantages of a method which facilitates the detection of the youngest intracorpuseular forms of all varieties of malaria will also be obvious. With regard to the more mature intracorpuseular forms the examination of moist films or staining by any of the methods in general use demonstrates these with sufficient certainty, but with inferior detail of structure as compared with the method under consideration. It is, however, quite otherwise with the youngest forms. In illustration of this it may be mentioned that in two of the cases recently invalided from India, both markedly cachectic and liable to atypical attacks of pyrexia at irregular intervals, numerous observations of their blood by the ordinary methods yielded a constantly negative result, except that on one occasion a single crescent was detected. On the other hand, films made at the same moment, but stained in the manner described below, demonstrated the presence of numerous young undotted parasites in both cases; indeed, in one of them these were so abundant that a careful count was made, with the result that no less than 8 per cent. of the red cells were found to be infected, many having two distinct parasites (Fig. 4). Figs 1-4 and 24 were drawn from this case, and show very young ring-formed quartan parasites, and were selected to show the most usual disposition of the small nucleoli of the parasite, which are deeply stained by this method. In the great majority of young forms there are two of these nucleoli, and they are invariably situated in the wall of the parasite and, most commonly, directly opposite to one another. Oval or pyriform rings are common, while the shape of the free amœbula, shown in Fig. 24, which is apparently attacking a normal red cell, is a frequent type.

Another point which this ready detection of young forms has enabled me to note is that in every case in which the parasite was found to be present during or immediately preceding an attack, an examination of the patient's blood at any period of the attack or between the attacks has given a similar positive result, though quantitatively less, while by other methods, applied simultaneously, negative results were recorded on many occasions.

It was also noted in several cases that these young forms, though at times very numerous, did not reach maturity, and in such cases one or two red-stained dots were often seen lying on or in red cells apparently unaffected by parasites. These dots recalled the nucleoli figured in 1-4, and may possibly have been the remains of a partially-destroyed parasite. These cases were at the time being treated with quinine.

There are many other points of great interest in connection with the persistence of these young immature forms in the blood and their bearing upon prognosis and upon the effects of quinine and other remedies, but the number of my observa-

tions is at present insufficient to generalise from; and it is in the hope that others, more favourably situated with regard to clinical material, may be led to try this method of staining that I have been led to give a description of its technique.

Thirdly, in the detection of the "mixed" forms of malarial infection great help is obviously to be had from any method by which one variety is marked out sharply and definitely from the others. The presence of Schüffner's dots in the red cells infected by the tertian parasite (Figs. 5-23) does for this variety of malaria what the presence of crescent forms does for the variously named remittent, pernicious, or æstivo-autumnal, and, if we accept the classification which appears to find most favour at the present moment, there remains but the quartan in which the proved absence of crescent forms or of infected red cells containing Schüffner's dots suffices to distinguish it from the others. The criteria at our command for the differential diagnosis of the varieties of malarial fever, other than this selective staining reaction—namely, the clinical characteristics of the fever, the size of the infected red blood corpuscles, the darker or lighter shade of the melanin granules, the character of the amœboid movements of the parasite, and the number of spores contained in the mature sporocyte—form a collection of points which it is far from easy to determine for every case, and which involve the preparation and examination of both fresh and dry films at several stages of the attack. On the other hand, as far as my observations go, Romanowsky's stain rarely fails to demonstrate the nature of the parasite or the existence of a mixed infection on a single examination of a properly prepared and stained film. Of the cases of malaria at Netley on which I have tested this method the larger number proved to be mixed infections—as, indeed, one would expect from their atypical clinical features and their chronic and intractable nature—and, of these, the most frequent combination was that of the tertian and the pernicious forms.

In proceeding now to a description of the preparation and application of Romanowsky's stain for the purpose of demonstrating the presence and nature of malarial infections of the blood the method described by Maurer has been fairly closely followed, and, in the points in which the technique described below differs from his, it is not to be understood that I consider these departures better, but that they, I trust, render the process somewhat easier of application and more capable of being carried to a successful issue by those whose microscopical outfit is limited.

PREPARATION OF THE BLOOD FILM.

A series of cover glasses, well polished and perfectly free from grease, are laid on a clean sheet of paper close to the patient, and blood films prepared as follows: Care should be taken that the cover glasses are never subsequently handled except with a pair of forceps, as the slightest trace of moisture from the fingers will endanger the result. Any method which ensures a thin even film may be used, but perhaps the easiest is the following: A few strips of very thin tissue paper—cigarette paper answers admirably—are cut into rectangular slips 2 inches by $\frac{1}{2}$ inch. A drop of blood the size of a pin's head is expressed from a needle prick in the patient's finger and lightly touched by the end of one of the paper slips. The edge of the paper bearing the droplet of blood is then quickly applied to a cover glass and when the drop has spread itself out between the glass and the paper the latter is gently and lightly drawn across its surface. This will be found to leave a very thin and evenly-distributed film. Unless pains are taken by this or some similar method to secure a thin even film, in which the red blood corpuscles are well spaced out and not overlying one another, good results are not to be expected. A series of films having been prepared in this way are allowed to dry in the air, or the drying may with benefit be hastened by gentle warming.

Fixing.—Immerse the films for from two to five minutes in a mixture of equal parts of ether and absolute alcohol, or, as an alternative if ether be not available, as may happen in a hot climate, in absolute alcohol alone for ten minutes. Fixing solutions containing perchloride of mercury are unsuitable, while fixation by heat is troublesome and presents no advantages over the first method. After removing the film from the fixing solution, wash it well in water and allow it to dry in the air before staining. The shorter the intervals

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between the preparation and fixation of the films and between the fixation and the staining the better are said to be the results, but I have found that unfixed films two months old may be successfully stained by slightly increasing the period of immersion in the dye. In any case, films must not be fixed until immediately before they are stained.

Staining.—The effects produced by Romanowsky's staining are due to a red dye which is extracted from alkaline solutions of certain kinds of methylene blue when to such a solution is added a small quantity of a very weak watery solution of eosin, and the evidences of the advent of this red dye are found in the appearance on the surface of the mixture of a brilliant metallic scum, a certain amount of precipitation and the staining of the walls of the containing glass vessel, or of any glass object dipped in the mixture, of a definitely red tint. This red dye is called by German authors "Rot aus Methylenblau," and in its extraordinary affinity for nuclear chromatin lies its great value as a differential stain for the malaria parasite, and also for demonstrating the structure of bacteria. That it is a new product, evolved by the interaction of the eosin and methylene blue and is not due to the weak eosin itself, is easily seen by the failure of its appearance when an ordinary watery solution of methylene blue is substituted for the special alkaline solution of which the formula is given below. Only a few varieties of methylene blue will produce this red dye in sufficient quantities, and Maurer gives a list of those with which he has obtained the best results, as well as details of his method of titrating one against the other so as to obtain the maximum staining power; but for the purpose of this article it will suffice to describe only the solutions with which I have worked, and which have yielded equally satisfactory results in other hands in this laboratory. Both the dyes described below were obtained from the firm of Dr. G. Grüber and Co. (Leipzig). Should these particular dyes be unprocureable, the proper proportion of eosin to be added to the solution of methylene blue must be determined by experiment.

Solution A.—"Medicinal" methylene blue. A 1 per cent. solution of this is made in distilled water, and then rendered alkaline by the addition of 0.5 per cent. of sodium carbonate. It is necessary before use that this solution should be heated for some time, and this may be conveniently done by leaving it in a tropical sun for two days or in a warm room for a week. To prevent the growth of moulds 0.25 per cent. of formalin may be added. It is recommended that after heating it should be allowed to stand for a week or two before use.

Solution B.—"Eosin extra B.A." A 1-1,000 solution in distilled water.

These concentrated stock solutions A and B, preserved in stoppered glass bottles, will keep indefinitely. When fixed blood films are to be stained, a portion of each of these solutions A and B is further diluted with distilled water in the proportion of 1 to 25, and kept ready in a couple of graduated glasses. An equal volume of each diluted solution is now poured on to the cover glass in such a way that they come in contact with the film at the moment of mixing. The reason for so doing is that it is at this moment that the production of the red dye is greatest and that it acts with most intensity. A convenient way of carrying this out is to pour a measured quantity of each—say 2 c.cm.—into two watch glasses, the cover glass being placed, film uppermost, in a third empty watch glass. The glasses containing the diluted A and B solutions are then picked up in either hand and their contents poured simultaneously upon the film. To ensure intimate mixture of the two solutions, the cover glass is now picked up with a pair of forceps and used to stir the mixture; it is then replaced, film downwards, at the bottom of the watch glass, and allowed to stain at room temperature for from half to one hour. Longer staining than this or heating are undesirable, and add nothing to the clearness of the result.

At the end of half an hour the film is removed from the stain and examined in water under a power of $\frac{1}{2}$ or $\frac{1}{4}$ inch, with a view to seeing whether the red dye has acted sufficiently intensely. The index to this is found first in the leucocytes, whose nuclei should appear sharply defined and stained an intense ruby-red colour; secondly, in the blood plates, which will at once attract attention, being stained sharply and of the same deep tint as the nuclei of the leucocytes. If instead of this deep-red colour the nuclei and blood plates are only purple or deep violet, the staining is insufficient, and the films must be replaced in the solution for a short time. A certain amount of deposit will also have

taken place, but this is easily got rid of subsequently in the process of decolorisation.

The point to which I have indicated the staining should be carried represents a slight degree of overstaining, as it is simpler to overstain and then decolorise than to stop the action of the dye at the right moment. Several methods of carrying out this decolorisation have been advocated, of which two are at the same time simple and efficacious. First, rapid method; the film after washing in water is rinsed in absolute alcohol for two or three seconds, then immediately transferred to water and all traces of alcohol removed. Second, slow method; in this water alone is used and the decolorisation may be carried out either by holding the film under a tap of running water for some time, or by leaving it to soak in water for from half an hour to one hour. In either case the extent of the decolorisation is controlled by examination under the microscope from time to time. Whichever method be adopted the appearance of the film when it is ready to mount should be as follows: The red cells are transparent and nearly colourless, or perhaps slightly tinged green or pink. The polynuclear leucocytes have their nuclei stained ruby red or magenta, the extranuclear portion being colourless or containing a few reddish granules. The nuclei of the mononuclear leucocytes and lymphocytes are of the same colour as those of the polynuclears, and their margins are very clearly defined, while the extranuclear portion is stained a beautiful eau-de-nil or pale blue colour. The blood plates are ruby red, with definite involution margins.

Mounting.—The films are now dried in the air or by gentle heat, and mounted for examination. Unfortunately, Canada balsam has not proved a very good mounting medium, as it detracts from and alters to a considerable extent the brightness of the colouring described above, and I have not, so far, been successful in finding a suitable substitute. However, either it or cedar oil may be used for the purpose without interfering with the results other than those of an æsthetic nature.

In conclusion, I wish to express my thanks to Civil-Surgeon G. H. B. Harvie, M.B., for the assistance he has afforded me in the clinical examination of many of these cases.

REFERENCES.

¹ Die Tüpfelung der Wirtszelle des Tertianparasiten, Dr. Georg Maurer, *Centrabl. für Bakt.*, vol. xxviii, Nos. 4 and 5. ² Beitrag zur Kenntniss der Malaria, Dr. W. Schüffner, *Deutsch. Archiv f. Klin. Med.*, vol. lxix.

DESCRIPTION OF COLOURED PLATE.

- Figs. 1-4.—Young intracorporeal forms of the quartan parasite.
Fig. 1.—Circular ring-form with single nucleolus.
Fig. 2.—Oval form with two nucleoli situated laterally.
Fig. 3.—Oval form with two nucleoli situated at the poles.
Fig. 4.—Red cell infected with two parasites.
Figs. 5-7.—Young forms of the tertian parasite showing gradual enlargement and alteration of the shape and position of the nucleolus; also the presence of Schüffner's dots throughout the red cell, even in the youngest form—No. 5.
Fig. 8.—Red cell infected by two young tertian parasites.
Figs. 9-12.—More fully-developed tertian parasites, in 10 and 11 showing amoeboid processes.
Figs. 13-23.—Various forms of tertian parasites approaching maturity, taken from another case, and showing more numerous and finer dots; also enlargement and altered contour of the infected red cells. In Figs. 15, 22, and 23 the enlarged nucleoli are apparently dissociated from the parasite, and free in the substance of the red cell.
Fig. 24.—A free amoeba attacking a healthy red cell.
Figs. 25 and 26.—Two stages in the formation of the mature quartan sporocyst or rosette.
Fig. 27.—Crescent form of pernicious malaria showing the melanin granules almost masked by a mass of nuclear chromatin.

PRESENTATION.—Dr. F. W. Kidd, whose term of office as Master of the Coombe Lying-In Hospital, Dublin, ended in December last, has been presented with a handsome gold watch as a token of the regard and esteem in which he was held by the staff, students, and nurses associated with him during his period of mastership. The presentation was made by Dr. Scully, ex-Assistant Master, and Dr. Kidd, in thanking the subscribers, dwelt on the pleasure it afforded him to know that past students from distant parts of the world had given their approval to the presentation, for amongst the most pleasant of his hospital recollections was that of the happy association he had experienced "with a large number of representatives from almost all parts of the globe who had spent various periods in the hospital."