NOTES ON MALARIA

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From the Pathological Laboratory of the King George Hospital, July, 1917.

PART I.

The intravenous route in the treatment of the active disease; technique employed and résumé of results obtained in fifty cases in which quinine bi-hydrochloride was given intravenously.

ADDENDUM.

Record of a case in which, during an afebrile period, tartar emetic was given intravenously.

PART II.

Incidents in the life-cycle of the parasites. (Plate.)

(a) Certain forms, or combinations, in the endogenous cycle of *Plasmodium vivax*; critical examination of the evidence put forward by Schaudinn in support of the so-called parthenogenesis theory to explain relapses; other views as to the manner in which relapses may be brought about.

(b) Initial stages in the exogenous cycle of *P. falciparum*; maturation of crescents, both male and female.

1 This article was received in July, 1917.
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INTRODUCTORY.

The following notes are based on observations and preparations made during the past nine months in the wards and pathological laboratory of the King George Hospital during the ordinary routine of general work. These observations have been rendered possible by facilities accorded by Lieutenant-Colonel R. C. Cottell, R.A.M.C., the Officer in Command, and by help received from various colleagues and from members of the nursing staff.

The patients admitted for malaria were mostly from Salonika, some were from Mesopotamia, and two were from Africa (one from the West Coast, and one from the East). In a few instances malarial relapses occurred in patients who had been admitted on account of wounds, or of other sicknesses. In upwards of a hundred cases in which parasites were found the parasite was that of quartan in one case only, of malignant tertian in nineteen, and of benign tertian in the remaining cases. The distribution in time was very uneven, as almost all the cases of malignant tertian were admitted in October of last year. In one case the parasites of both malignant and of benign tertian were present in the blood at the same time; and in a few others, in which "rings and crescents" were reported to have been found in Malta, only parasites of benign tertian were found while the patients were in the King George Hospital. Among the cases of benign tertian the presence of more than one generation of the parasite was perhaps the rule. Parasites belonging to different generations were frequently seen in the peripheral blood at the same time, and it was not unusual to find two generations equally active, separated in time by twenty-four hours, and giving rise to a quotidian type of fever. In some the number of red cells attacked was very large, and as many as six ring forms of the parasite have been seen in a single red cell. With two exceptions all were cases of relapse—the exceptions being cases of benign tertian that developed symptoms while the patients were under surgical treatment. One of these patients had been wounded in Mesopotamia, the other on the Struma front; but, so far as they knew, they had their first symptoms of malaria in the King George Hospital. The first case ran a quotidian type of fever for some time before its true nature was suspected. The second case was seen and treated during the first paroxysm, and will be referred to again in dealing with certain forms or combinations in the endogenous cycle of P. vivax under Part II.
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I.—The Intravenous Route in the Treatment of the Active Disease.

The use of the intravenous route for the administration of quinine in malaria has hitherto been recommended almost exclusively for emergency cases, such as “pernicious comatose remittent,” where rapidity of action is all-important. But the intravenous route has other and quite special advantages to recommend it in the treatment of malaria. It is the only route by which one can concentrate on the parasite in the circulating blood so as to obtain, within therapeutic limits, the maximum effect at the optimum time. Provided, then, that a safe and convenient technique can be recommended, the use of this route should be extended to many more cases of the active disease. In view of the importance of the subject at the present moment, I venture to explain a technique that I have found to be both safe and convenient, and to give a résumé of results obtained in fifty cases of malaria in which quinine bi-hydrochloride was given intravenously during the active stages of the disease.

Solution Used.—The strength of the solution used is twenty per cent. An ounce of quinine bi-hydrochloride is dissolved in, say, 120 cubic centimetres of normal saline, and the volume of the solution is then brought up to 160 cubic centimetres by the addition of more saline. The solution is heated until it boils, and it keeps well in a glass-stoppered sterile bottle protected from direct sunlight. If not absolutely clear, the solution is passed through a sterilized cotton-wool filter, and each dose is again brought to the boil before being used. One cubic centimetre of the solution contains three grains of quinine bi-hydrochloride.

Technique Employed.—Before explaining the technique employed, I think it desirable to refer to a paper by MacGilchrist, entitled “Quinine and its Salts: Their Solubility and Absorbability.” A summary of this paper appeared in Paludism (1911). My attention was called to it by Lieutenant-Colonel S. P. James, I.M.S., and its contents are probably familiar to members of the Indian Medical Service, and to others interested in the treatment of malaria. I refer to it now as I suspect that it may have unduly influenced some against the use of solutions of such strength as renders the intravenous administration of quinine generally

1 The samples of quinine bi-hydrochloride supplied varied; and only those giving a clear solution were used. With some samples, the solution was more acid than with others, and in these cases the acidity was reduced by the addition of about 0·25 per cent soda bicarb.
practicable. Captain MacGilchrist considers that "quinine salts in the dilutions usually employed are quite unsuitable for hypodermic use," and that "this mode of administering quinine (subcutaneous including intramuscular) should be abandoned." His conclusions about the intravenous route as given in the summary referred to are as follows: "Great dilution (at least 1 in 150) is necessary in order to avoid the dangers special to quinine. Seven grains of quinine bi-hydrochloride should be dissolved in two or three pints of saline. This mode of administration is called for in cases of emergency, as in pernicious malaria with coma or other cerebral symptoms; and in such cases the extreme dilution affords the additional advantage of attenuating the toxins and favouring their elimination. In this dilution intravenous injections of quinine are quick and sure, and their action is not fleeting. They are attended by no risks peculiar to quinine, but only by those attendant on intravenous infusions generally. When there is a tendency to hemoglobinuria the quinine alkaloid, instead of one of its salts, should be used."

Accepting the statement that "great dilution is necessary in order to avoid the dangers special to quinine," I believe that such dilution can be obtained in the circulating blood by attention to technique, even when comparatively strong solutions are used for injection. The points I would emphasize are: select a good-sized

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1 Captain MacGilchrist's experiments relating to miscibility of quinine with blood serum apply chiefly to hypodermic and intramuscular injections. With proportions of serum much larger than he used, other results are obtained. Blood plasma in vivo is not the same as blood serum in vitro, but on mixing together a 20 per cent. solution of quinine bi-hydrochloride in normal saline with human blood serum in test-tubes in the proportions given below, and placing them in the incubator at 37° C., the results were as follows:

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>After 1 min.</th>
<th>At end of 10 mins.</th>
<th>At end of 1 hour</th>
<th>Overnight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 in 2</td>
<td>Clear; fluid</td>
<td>Opalescent; jelly; like</td>
<td>Opalescent; solid</td>
<td>Opalescent; solid</td>
</tr>
<tr>
<td>1 in 5</td>
<td>Opalescent; fluid</td>
<td>Milky; fluid</td>
<td>Milky-white; solid</td>
<td>Milky-white; solid</td>
</tr>
<tr>
<td>1 in 10</td>
<td>Slightly opalescent; fluid</td>
<td>Opalescent; fluid</td>
<td>Opalescent; fluid</td>
<td>Opalescent; viscid</td>
</tr>
<tr>
<td>1 in 20</td>
<td>Less opalescent; fluid</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Opalescent; fluid</td>
</tr>
<tr>
<td>1 in 40</td>
<td>Very slightly opalescent; fluid</td>
<td>Slightly opalescent; fluid</td>
<td>Slightly opalescent; fluid</td>
<td>Slightly opalescent; fluid</td>
</tr>
<tr>
<td>1 in 80</td>
<td>Trace of opalescence; fluid</td>
<td>Very slightly opalescent; fluid</td>
<td>Very slightly opalescent; fluid</td>
<td>Very slightly opalescent; fluid</td>
</tr>
</tbody>
</table>

In higher dilutions, 1 in 160, 1 in 320, etc., there was no change, the mixture remaining quite clear and fluid after being in the incubator overnight. Micro-
vein, use a sharp fine-bore needle, be sure that its point is well in the lumen of the vein, and inject the solution very slowly. The details, many of which, of course, may be modified by the individual operator, are as follows: Five or six cubic centimetres of the stock solution are brought to the boil in a test-tube. The patient is in bed, and lies on his back. The median basilic or other large superficial vein at the bend of the elbow is selected and the skin over it is painted with tincture of iodine. While this is drying, the quantity of solution to be injected is drawn from the test-tube into a sterile five-cubic-centimetre syringe fitted with a sharp sterile needle. When the syringe is charged with the solution a little sterile saline may be drawn into the needle. The nurse or assistant now grasps the patient's arm just above the elbow, lightly, but so as to compress the veins. If the patient now forcibly closes and opens his hand two or three times in succession, the veins at the bend of the elbow will stand out prominently. The secret of clean and easy puncture is a sharp needle and a prominent vein. When the needle enters the vein the pressure above the elbow should be removed. If the bore of the needle used is not very fine, and if pressure is not immediately removed, blood is apt to regurgitate into the syringe, and that should be avoided. On the other hand, a little blood should reach the nozzle (if necessary, be drawn into it) to show that the needle is in the vein. With the point of the needle well in the lumen of the vein the operator should maintain complete control over the injection. A needle of fine bore makes slow injection easy. From fifteen to twenty seconds should be occupied in injecting one cubic centimetre of the solution and a pause of some seconds made between each cubic centimetre. If this is done, one or two rounds of the circulation will have been completed before a full dose is given. Singing in the ears and other symptoms due to the action of quinine begin usually by the time that six to nine grains of the quinine bi-hydrochloride have been injected. The operator should so control the injection that he can pause or stop at any stage he pleases. I have not yet had occasion to give less than nine grains, nor have I ever given more than fifteen grains of the bi-hydrochloride at one time. Before withdrawing the needle a little blood may be drawn into its bore to replace and so to

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 scopically examined the 1 in 10 and 1 in 20 dilutions seemed to be emulsions of fine amorphous granules, probably of albumin or other protein of the serum. In the 1 in 40 dilution, in addition to fine amorphous granules, fine needle-like structures (probably quinine) about ten microns in length, and a fraction of a micron in width were seen.
prevent any leakage of the quinine solution into the tissues; but, with a needle of fine bore this is unnecessary. As the needle is withdrawn, the patient’s arm is slightly raised and no blood escapes. The puncture may be touched with tincture of iodine, but no dressing of any kind should be necessary. The requirements are about the same as for an intramuscular injection, and the process is much less painful to the patient. If the injection has been strictly intravenous no local reaction follows. In considerably over 100 injections I have only once seen local trouble, and that followed on hypodermic rather than on intravenous injection. A small area of thin skin necrosed and secondary infection through the necrosed area set up an acute cellulitis. The vein was not involved. The cellulitis subsided and ended with the discharge of seropus and a few connective tissue sloughs, after which healing was uneventful. In other two cases, when using a rather acid solution a mild subacute local phlebitis followed, but this was scarcely sufficient to attract the patient’s attention, and entirely subsided in two or three days. One was a cardiac patient with unhealthy vessels, and in the other the injection had been made into a rather small vein in the forearm. As regards general reaction, if the injection is kept well under control, and if the solution is injected very slowly with frequent pauses, there should be no symptom to cause the slightest anxiety. A nervous patient may show signs of hysteria, even to the “globus hystericus,” while the injection is being made, but if the pulse remains good there is no cause for alarm. Special precaution should be exercised in dealing with cardiac cases in some of which intravenous injection may be altogether contra-indicated; but in general, in these and in other cases the special precautions are concerned with dosage and with individual idiosyncrasy, and apply equally to other modes of administering quinine.

Résumé of Results obtained in Eighteen Cases of Malignant and in Thirty-two Cases of Benign Tertian in which Quinine Bi-hydrochloride was given Intravenously during the Active Stages of the Disease.

The results of the injections on the patients were noted clinically, and the effect on the parasites was observed by examination of blood films.

(A) Malignant.—Of the eighteen cases all but three had taken quinine prophylactically for longer or shorter periods before the first attack began. Some of the earlier cases, admitted in October,
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1916, continued to have remittent fever and to have "ring" forms of *P. falciparum* in their blood, notwithstanding that they had taken large quantities of quinine and were still taking (some days after admission) ten grains of the hydrochloride in solution every eight hours by the mouth. The bowels had not been neglected, and the patients had been in bed since admission. In these cases the exhibition of quinine by the mouth was stopped for a day, and, in place of it, fifteen grains of the bi-hydrochloride were given intravenously in the manner already described. The results were so satisfactory that thereafter every case of remittent fever in which "ring" forms of *P. falciparum* were found in the peripheral blood was given fifteen grains of quinine bi-hydrochloride intravenously as a preliminary to further treatment. Altogether eighteen cases of "malignant tertian" were treated in this way, and the results of the injection were the same in every case. The attack was broken. The patient on the day following the injection invariably expressed himself as feeling very much better, his temperature had fallen to below the normal line, and "ring" forms of the parasite, though frequently present in very small numbers on this day, could not be found by the end of the second day in films of the peripheral blood. On the day following the injection, quinine was begun, or continued, by the mouth; and ten grains of the hydrochloride in solution every eight hours were now sufficient to keep the temperature down, even though similar doses may have failed to reduce it before the injection was given. Although "ring" forms of the parasite disappeared from the peripheral blood, crescents remained as before. In one case, after an attack had been broken by the intravenous injection of fifteen grains of quinine bi-hydrochloride, no quinine was given by the mouth, but intravenous administration was continued for three days longer—nine grains on the second, twelve grains on the third and on the fourth days—and the blood was examined for crescents up to a week after the last injection had been given. On each occasion crescents were found. A day or two later (nine days after receiving the last injection) the patient had a relapse. I saw him soon after it began. His temperature was then just over 100° F., he was vomiting bile, and many "ring" forms of the parasite, together with a few crescents, were found in a film of blood taken from his finger. After finding the "rings" no time was lost before giving him an intravenous injection containing fifteen grains of quinine bi-hydrochloride. Some hours later he felt quite comfortable. He had a good night, and next morning he felt quite well,
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and his temperature was below normal. The attack was broken, and further treatment was now continued by the mouth. At the end of a fortnight one more intravenous injection containing twelve grains of quinine bi-hydrochloride was given, and after that a mixture containing iron, arsenic, and pux vomica was added to the treatment by the mouth. Crescents were still present in the peripheral blood at this time, but a fortnight later, i.e., a month after the relapse, none could be found in thick dehæmoglobinized films taken on four consecutive days. The patient had then been up for a week. He felt well, and looked very much better than he did when he was admitted. He was then transferred to a Convalescent Hospital. Being a dispenser in the Royal Army Medical Corps, the history he gives is interesting in its bearing on quinine administration:

His first attack began on October 1, 1916. For three months previous to this (from July 1 till the beginning of the attack) he had taken five grains of quinine sulphate in solution daily. The attack began with a rigor and with bilious vomiting. He was treated in hospital (in Salonika), and during each of the first four days he got ten grains of quinine intramuscularly and thirty grains by the mouth. Notwithstanding treatment, bilious vomiting, rigors and exacerbations of temperature continued daily for three successive days. He cannot say how long his temperature remained up, but bilious vomiting ceased after the third day. At the end of four days intramuscular injections were left off, but quinine by the mouth was continued for three weeks longer—thirty grains in solution daily. After this course of treatment he returned to duty, but, four days later, another attack began and he was sent back to hospital at once. This attack was practically a repetition of the former one. Bilious vomiting was pronounced, and, as before, continued for three days, in spite of treatment. He remained in hospital for a month, and, during that time, took thirty grains of quinine in solution, daily, by the mouth. He again returned to duty, but felt ill all the time, and at the end of three weeks he was sent to Malta. He remained in Malta from January 1 till March 12. During that time he had two attacks, each of which began like the two former attacks, and bilious vomiting continued as before for three successive days, in spite of treatment. While in Malta he took fifteen grains of quinine daily for seven weeks on end, and had then to discontinue on account of irritation of the stomach and deafness. On arrival in England he was sent to the King George Hospital. He was then emaciated and sallow. His spleen was palpable, and during the attack which began on March 27 was tender. As previously stated, both attacks which he had while in the King George Hospital began with shivering and bilious vomiting, just as previous
attacks had begun, but each was cut short on the first day by an intravenous injection containing fifteen grains of quinine bi-hydrochloride given soon after the attack began.

After being in convalescent hospital for nearly seven weeks this patient had a rigor and rise of temperature, followed by sweating, but he did not suffer from bilious vomiting or from sickness. He took quinine by the mouth. Two days later he had another similar paroxysm. He continued to take quinine by the mouth, and returned to the King George Hospital. I examined his blood on arrival, and found only gametocytes of benign tertian. When in this hospital before he went to the convalescent, no parasites of benign tertian were ever found in his blood, only those of malignant tertian.

Another case in which galyl was first given is worth referring to:

In this case a full dose of galyl was given intravenously by one of the physicians while "ring" forms of P. falciparum were present in the peripheral blood. I examined films of the peripheral blood daily for three days after this injection. Neither "ring" forms of the parasite nor the patient's temperature seemed to be influenced in the slightest degree, so at the end of three days I gave him twelve grains of quinine bi-hydrochloride intravenously. On the following morning his temperature was below normal, he felt well, and "ring" forms of the parasite had all but disappeared from the peripheral blood. In films taken on the day after no "ring" forms were found. The patient had an after course of treatment, beginning on the day after the injection, similar to that given to the case mentioned above. Up till three weeks from the day he had the intravenous injection of quinine and while still taking quinine by the mouth, crescents were found in the peripheral blood. Since then I have several times examined his blood for crescents without finding any. He has not yet had another relapse, and has steadily improved in condition, so that now (five months later) he feels and looks very much better.

(B) Benign Tertian.—The thirty-two cases of benign tertian chosen for intravenous injection were most of them severe cases. In many, parasites of two generations were about equally numerous in the blood at the same time giving rise to a quotidian type of fever. The great majority had had several relapses, and each had, since the first attack, taken large quantities of quinine. Some during former attacks had been given quinine intramuscularly as well as by the mouth. Most had had more than one course of hospital treatment, varying from three weeks to three months at
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a time—the longer periods including treatment in convalescent camp or convalescent home. A few had been in many hospitals and had had long courses of quinine by the mouth. Others had been having constantly recurring attacks, and these as a rule had not taken quinine during the afebrile intervals, but had taken it by the mouth up to thirty grains a day for five or six days at a time while fever lasted. All but six had taken quinine prophylactically for longer or shorter periods before the first attack. Five of these six had had many relapses and were severe cases; although the treatment they received from the time of the first attack did not differ from that received by others who had taken quinine prophylactically before their first attack.

To the majority of cases of benign tertian the quantity of quinine bi-hydrochloride given in each intravenous dose was twelve grains. This dose was arrived at as a result of comparing the effect on the parasites in different cases as ascertained by examination of blood films taken before and after doses of 9, 12, and 15 grains. By the examination of films in a number of cases in which the dose was repeated an endeavour has also been made to ascertain when and how often the dose should be repeated in any given case. In every case, the first injection was given during a paroxysm, usually at the end of the hot stage or just as the skin began to get moist and the temperature began to fall. If given at the beginning of the hot stage, the quinine would probably add to the patient's discomfort. In two cases I had an opportunity of giving the dose at the very beginning of the rigor. In both cases, according to the patients' statements, the rigor seems to have been shortened, while the hot stage was proportionately lengthened. In every case the first intravenous injection was sufficient to break the attack, so that the paroxysm next in order did not occur. This was so even in cases where two generations of the parasite were present in the circulating blood, and where a quotidian type of fever was to be expected. Unlike the crescents of malignant tertian, the gametocytes of benign tertian disappear from the peripheral blood under the direct action of quinine. But although quinine affects all stages of P. vivax present in the circulating blood, examination of films shows that all stages are by no means equally affected. In this connexion the following cases may be quoted:

(a) When only one generation of the parasite was seen to be active, the patient got in all three intravenous injections of twelve grains each.
Case 1.—In this case, the first injection was given during a rigor, and the second and third injections on alternate days when the two “rigors” next in order would have been due, had the case remained untreated. Examination of films:

(1) Films taken immediately before the first injection was given. Gametocytes (male and female) fairly abundant: some schizonts with nucleus dividing and others where schizogony is completed, seen; many young trophozoites present—some, disk-like, clinging to red cells, and other “ring” form.

(2) Film taken twenty-six hours after the first injection was given. A few gametocytes present. No other forms of parasites seen.

(3) Film taken forty-eight hours after first injection was given. No gametocytes nor other form of parasite seen.

(4) Film taken twenty-four hours after second and just before the third injection was given. No parasite seen.

Case 2.—In this case, the first injection was given at the end of the hot stage, and the second and third injections at the same time on alternate days. Examination of films:

(1) Film taken just before the first injection was given. Gametocytes fairly numerous: fully divided schizonts and many young “ring” trophozoites seen.

(2) Film taken just before the second injection (i.e., forty-eight hours after the first) was given. No parasite seen.

(3) Film taken just before the third injection was given. No parasite seen.

Case 3.—In this case, the first injection was given during the sweating stage, and the second and third injections on alternate days when the two “rigors” next in order would have been due had the case remained untreated. Examination of films:

(1) Film taken just before the first injection was given. Many young trophozoites, mostly “ring” forms, and a fair number of gametocytes (male and female) present. No other forms seen.

(2) Film taken just before the second injection (about forty-two hours after the first) was given. A few gametocytes (male and female) present, and one schizont seen.

(3) Film taken just before the third injection was given. No parasite seen.

(4) When two generations of the parasite were seen to be active, and a quotidian type of fever, reported or expected, each generation was considered separately, so that the patient got six intravenous injections, one on each of six successive days.

Case 4.—This case had been having constantly recurring attacks and was seen during the first paroxysm of a recurrence—about the end of the hot stage, 5½ hours after the rigor began. Examination of films:
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(1) Film taken just before the first injection was given. Many gametocytes (male and female), many young "ring" trophozoites and also many half-grown, actively ameboid trophozoites present.

(2) Film taken just before the second injection (about eighteen hours after the first) was given. Gametocytes (male and female) present, also a fair number of very young trophozoites, mostly disk forms, clinging to the surface of the red cells. Some half-grown trophozoites are also present, but most of these are solid-like, suggesting that they may be half-grown gametocytes. A very few are ameboid, and some are fragmenting.

(3) Film taken just before the third injection (twenty-four hours after the second) was given. No parasite seen.

(4) Film taken just before fourth injection was given. No parasite seen.

**Case 5.** This case had had daily paroxysms for eight days before admission.

(1) Film taken three-quarters of an hour before the first injection was given. Many gametocytes (male and female), many fully divided schizonts, a large number of young "ring" and of half-grown actively ameboid trophozoites present.

(2) Film taken just before the second injection (about twenty-three hours after the first) was given. A fair number of gametocytes (male and female), also a fair number of schizonts with nucleus dividing and some young "ring" trophozoites present. No half-grown ameboid forms seen.

(3) Film taken just before the fourth injection (about twenty-four hours after the third and forty-eight hours after the second) was given. One gametocyte and no other form of parasite seen.

(4) Film taken just before the fifth injection was given. No parasite seen.

The merozoites free in the plasma and the young disk-like forms clinging to the surfaces of red cells are probably more directly exposed to the action of the quinine, but the young "ring" trophozoites are perhaps equally vulnerable. The "ring," or rather the "hollow-sphere" form, giving a large absorptive surface in proportion to the mass of the parasite, is said to be (and probably is) an adaptation to quick nutrition and rapid growth, but it also renders this form of parasite more vulnerable to quinine. The half-grown actively ameboid forms are seen (Case 4 and Case 5) to be affected to a less degree than the small "ring" forms, and the gametocytes are affected least of all. Thus the action of quinine on the various forms of the parasite present in the circulating blood seems to be in direct proportion to their nutritive activity. The time to con-
centrate against the parasite, therefore, is during the paroxysm, when schizogony has taken place and before the young trophozoites have passed the "ring" stage. The period covered by schizogony varies in different cases, but, roughly speaking, it extends from the beginning of the rigor to quite the end of the hot stage. If quinine is given intravenously during the rigor no great quantity is likely to be eliminated before the end of the hot stage, so that theoretically it should have its maximum effect on the cycle if given then. On the other hand, very few of the young trophozoites will have passed the "ring" stage before the patient's temperature begins to drop, so that the patient's comfort will be considered without the parasite being spared if the first intravenous injection is given at the very end of the hot stage or just as the skin begins to get moist. Subsequent intravenous injections should be timed for what would have been the end of the rigors or the beginning of the hot stage in the paroxysms next in order had the case remained untreated. The large majority of these thirty-two cases of benign tertian were treated in this way, and after giving three intravenous injections for each generation causing, or likely to cause, paroxysms, a modified course of treatment with quinine and including iron, arsenic, and nux vomica, by the mouth was recommended for some weeks longer. I have not heard that any of these cases have relapsed, but even if none have, the time that has elapsed is too short to allow of any deduction being drawn from that circumstance. In some of the thirty-two cases, only one intravenous injection was given in order to break the attack, and quinine was then continued by the mouth. In other cases quinine was given intravenously only, and the results were compared with the reported results of former attacks, when quinine was given intramuscularly and by the mouth, or by the mouth only. In all these cases the results were very much in favour of the intravenous route. The first dose of twelve grains invariably broke the attack, the total quantity of quinine given was very much less than formerly, the patients' appetites improved, and they felt better than they had done under any former treatment. Some of these cases left the hospital within a fortnight and have not been heard from since, but two cases that had been having constantly recurring attacks before admission may be mentioned. These cases are here designated Case 6 and Case 7.

Case 6.—This patient had his first attack on July 28, 1916—two weeks after his arrival in the Struma Valley. He had not taken quinine before this attack. After the attack he was treated in Salonika for three
weeks, getting quinine by the mouth t.d.s. and was then sent to Malta. In Malta, from August 4 till 11, he got quinine in solution, ten grains t.d.s. From August 11 till September 1 he got altogether twenty intramuscular injections of quinine, ten grains in each, in addition to quinine by the mouth. On August 19 it is reported that "crescents" were found in his blood. He left Malta on October 13, and arrived in England on October 26. He did not have any quinine on the voyage, but on arrival was sent to hospital in Devonport, where he had a "rigor," and was treated with quinine by the mouth. After a week he was sent to a convalescent home, where he remained three months; had two or three "rigors" every week, he says, until the last fortnight. He was then moved to another convalescent camp, and during ten days there he had three paroxysms, two of them on consecutive days. From camp he was sent to hospital, where he got thirty grains of quinine in solution daily by the mouth (ten grains t.d.s.) for six days. On returning to camp he had two more paroxysms and was sent home on sick leave. While at home he had daily "rigors" for five days. During this time he took quinine in tabloid form ten grains daily, then in liquid form twenty grains daily. On returning to his depot he had four more paroxysms, and on his way home again he took ill in the train, and was brought to the King George Hospital. Examination of blood film showed that two generations of the parasite of benign tertian were active. He was given fifteen grains of quinine bi-hydrochloride intravenously at the beginning of the sweating stage, and ten grains on the following day, when the paroxysms due to the other generation of parasites would probably have occurred. No further treatment was given. Two days later no parasite could be found in films of the peripheral blood. The patient said he felt better than he had ever done under former treatment. His appetite and his general condition rapidly improved. He remained in hospital for twelve days, being up and helping in the ward during the last week. He left feeling well, but not long after I heard that he had another attack.

Case 7.—In this case, also, no quinine had been taken before the patient had his first attack, but treatment was begun immediately the attack occurred. In Malta, he says that he took quinine by the mouth every four hours night and day for five weeks on end. Before he came to the King George Hospital he had been having attacks which lasted four or five days at a time at intervals of about five days. He took no quinine during the intervals between the attacks, but took ten and twenty grains daily during the attacks. A week before he was brought to hospital suffering from a paroxysm, he had had an attack which lasted five days, and during those five days he took thirty grains of quinine in solution daily by the mouth (ten grains t.d.s.). Two generations of parasites were found present in his blood on admission. He got, in all, three intravenous injections of quinine bi-hydrochloride—fifteen grains
in each. The first was given during the sweating stage, the second forty-eight hours later, and the third twenty-four hours after the second. He left hospital feeling well, but I heard from him later that he had another attack, which began on the twenty-fifth day after he had received his last injection.

In considering recurrences, the possibility of multiple infection must not be forgotten. Thus in two cases that had recurrences while the patients were still in hospital, the recurrences took place on days that did not correspond with the forty-eight hours cycle of the generations that were attacked by the intravenous injections. The first, which we will call Case 8, had a severe rigor on the morning of April 22. The rigor began about 6 a.m. At 8 a.m. his temperature was 106.4° F. He got an intravenous injection of twelve grains quinine bi-hydrochloride at 10.50 a.m. His skin was then moist and his temperature was 105.4° F. On April 24, at 10.30 a.m. he got another injection of twelve grains, and this was repeated about the same time on April 26. After the first injection he did not have a paroxysm until May 11. The rigor began about 11 a.m. This attack was at once cut short by an intravenous injection of twelve grains of quinine bi-hydrochloride given at the end of the hot stage or just as the skin began to get moist. Had this attack been a recrudescence the 10th or the 12th would have corresponded with the last attack, but not the 11th of May. It might be argued that in this case the fever either anticipated or postponed, but the hypothesis that the recurrence was a relapse due to a generation that was quiescent when the patient was treated for the preceding attack cannot be ruled out, especially as more than one generation of parasites were now seen in the blood film examined. In Case 9, in addition to many "ring" forms, one or two half-grown amoeboid trophozoites were seen in films of the peripheral blood taken just before the first intravenous injection was given. On April 20 this patient had a rigor which began just before noon. He got twelve grains of quinine bi-hydrochloride intravenously at the end of the hot stage, and this was repeated about noon on the 22nd and again on the 26th. He did not have a paroxysm after the first injection until May 11. The rigor on May 11 began about noon. In this case the recurrence was probably caused by the generation represented by the one or two half-grown trophozoites seen in the film taken just before the first intravenous injection was given. In a third case, one already referred to (p. 387), there can be no doubt whatever about the recurrence being a relapse due to a generation that was
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quiescent when the patient was treated for the preceding attack, because in this case the parasite causing the relapse was that of benign tertian, while in the preceding attack it was that of malignant tertian.

Case 9, referred to above, is interesting from another standpoint. He had a very large spleen extending to beyond the umbilicus and filling up a large part of the left flank. His first attack of malaria occurred in September, 1916. He had taken quinine prophylactically throughout the previous summer, and remained on duty after his first attack until towards the end of December, when he was sent to hospital suffering from pains in the bowels and enlarged and tender spleen. In Malta, no malarial parasites were found in his blood, and the question of kala-azar and splenic puncture was raised. Splenic puncture was not done. In hospital here, before he had the rigor on April 20, he had been taking quinine irregularly by the mouth and his temperature was irregular. During this period I found parasites of benign tertian in his blood on three occasions. He had an impression that quinine was responsible for all his troubles; but after the first intravenous injection, given on April 20, he was quite keen to have another. At this time, apparently, he did not realize that it was quinine that was being injected, for when the next attack began on May 15 he was very much averse to quinine but was quite eager to have the "injection."

SUMMARY AND CONCLUSIONS.

(1) The intravenous route has special advantages in the treatment of malaria during active (as distinguished from quiescent) periods of the disease. By this route, and at these times, the full quantity of quinine given can be concentrated against the parasite at the moment when it is most susceptible to its action; and the maximum effect of the drug, within therapeutic limits, can thus be obtained.

(2) By attention to a simple technique, quinine bi-hydrochloride in twenty per cent solution can be safely and conveniently given intravenously up to fifteen grains of the salt for a dose—a five-cubic-centimetre syringe and a suitable hypodermic needle being the only special apparatus required.

(3) In eighteen consecutive cases of malignant tertian with remittent fever and with ring forms of P. falciparum present in the peripheral blood, a single intravenous injection of fifteen grains of quinine bi-hydrochloride in twenty per cent solution was sufficient to break
the attack in every case; and, once the attack was broken doses of quinine by the mouth which had been insufficient to reduce the temperature were now sufficient to keep it down. Ring forms of the parasite quickly disappeared from the circulating blood after the injection, but crescents were not directly affected.

(4) In all these cases of malignant tertian with remittent fever the intravenous injection was given as soon as ring forms of \textit{P. falciparum} were found in the peripheral blood, i.e., as soon as the true nature of the fever was known.

(5) In thirty-two consecutive cases of benign tertian a single intravenous injection of twelve grains of quinine bi-hydrochloride in twenty per cent solution given during a paroxysm was sufficient to break the attack so that the paroxysm next in order did not occur. This was so even in those cases where a quotidian type of fever was to be expected.

(6) All stages of the schizogonous cycle of \textit{P. vivax} present in the circulating blood were directly affected by the injection, but not to the same extent. The young forms before they pass the ring stage were most affected, then the actively amoeboid forms, and others apparently in proportion to their nutritive activity. Gametocytes were the last forms of the parasite to disappear from the peripheral blood.

(7) The mature gametocytes of \textit{P. vivax}, unlike those of \textit{P. falciparum}, disappear from the peripheral blood under the direct action of quinine bi-hydrochloride given intravenously.

(8) In cases of benign tertian, the patients' comfort will be considered without the parasites being spared if the first intravenous injection be given at the very end of the hot stage. Subsequent intravenous injections should be timed to be given at what would have been about the beginning of the hot stage in the paroxysms next in order had the cases remained untreated.

(9) The intravenous injection of a twenty per cent solution of quinine bi-hydrochloride in normal saline in the doses and at the times stated can be relied upon to break promptly an attack of malaria; and, although immediate sterilization or even sterilization after any single course of treatment in cases that have already relapsed several times is not to be expected, it is reasonable to suppose that, if each relapse is cut short on the day of onset, the patient will stand a much better chance of ultimate sterilization; and that the shorter the time that the parasite is allowed to go on multiplying the less will be the risk of secondary changes due to the
activity of the parasite, and the greater will be the chance of quick and complete recovery.

Addendum.

Record of a case, in which, during an afebrile period, tartar emetic was given intravenously:

Rogers (1917) tentatively suggested that after the malarial paroxysms had been checked by quinine, "tartar emetic should subsequently be given intravenously, in the hope that it may prove of value in destroying the extracorporeal stages of the malarial parasites, and so prevent relapses, and greatly lessen the infectiveness of the patient to malarial-bearing mosquitoes, by killing the crescents of the malignant tertian variety and the corresponding resisting forms of the other types of malaria."

On February 28, 1917, a patient who had suffered from malaria was given six centigrammes of tartar emetic intravenously during an afebrile period when crescents were the only forms of parasite present in films of his peripheral blood. Films taken thirty-six hours afterwards showed quite as many crescents as did those taken just before the injection was given. Crescents were never very numerous, but could easily be found in thick dehæmoglobinized films, and, by searching, one or two could always be found in ordinary thin films. For some days after the injection his blood was examined daily, and crescents were found on every occasion. On March 9, i.e., on the ninth day after the first intravenous injection of 'tartar' emetic, a second injection containing eight centigrammes was given. In a film taken immediately before the second injection was given, crescents were found as before; but in addition, gametocytes and trophozoites of the parasite of benign tertian were now present. This was the first indication we had that the patient had suffered from a mixed infection—from benign as well as from malignant tertian. On March 10, crescents were still present as before, and the parasites of benign tertian had increased in numbers. On March 11, he felt slightly chilly, and

1. In the same communication Rogers speaks of the extracorporeal cycle "which," he says, "is responsible both for the frequent relapses of the ague and . . . ." It is evident that by "the extracorporeal stages of the malarial parasites" and by "the extracorporeal cycle" Rogers means the gametocytes as present in the blood; but the term extracorporeal as applied to them is quite inaccurate; and his definite statement that they are responsible for relapses will be looked upon by many who consider that this is still "sub judice" as being too arbitrary.
ripe schizonts in various stages of schizogony up to fully divided forms were seen, along with young trophozoites and with gametocytes of benign tertian as well as with crescents, in the films taken at this time.

In this case, then, if one allows for the incubation period, it would appear that the first dose of six centigrammes of tartar emetic given intravenously, in place of killing crescents, produced or hastened on conditions favourable to the onset of a relapse of benign tertian whose latency had not previously been suspected; while the second dose of eight centigrammes, given nine days after the first, neither arrested the development of the relapse of benign tertian nor did it act injuriously on the crescents.

II.—INCIDENTS IN THE LIFE-CYCLE OF THE PARASITES. (PLATE.)

(a) Certain forms, or combinations, in the endogenous cycle of P. vivax: critical examination of the evidence put forward by Schaudinn in support of the so-called parthenogenesis theory to explain relapses; other views as to the manner in which relapses may be brought about.

It is not yet known in what way, or in what form, the parasite survives the long periods that may elapse between relapses. There has, nevertheless, been a good deal of speculation as to the manner in which relapses are brought about. The view that undoubtedly has held the foremost place for the longest time is what is best known as Schaudinn’s parthenogenesis view. Grassi had suggested the possibility of explaining relapses by parthenogenesis of gametocytes, but it was Schaudinn (1902) who is supposed to have demonstrated its actual occurrence, and to have proved that this is the normal manner in which relapses are brought about. This view assumes that only female gametocytes (or sporonts) survive the healthy intervals between relapses. They are supposed to maintain their existence in a “resting state”—a state that is normal to them while they remain in the vertebrate host—if not in the peripheral blood, which is their natural place when fully grown, then somewhere in the deeper organs; but under certain imperfectly known conditions that favour a relapse they are supposed to “multiply parthenogenetically and produce a brood of merozoites, which are the starting point of a fresh series of schizogonous generations.” No serious examination of Schaudinn’s argument and of the evidence he adduces in support of this view has, apparently, been undertaken. Harrison (1909) published a paper
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accompanied by a coloured plate supposed to support the parthenogenesis view, but I mention it only to give the reference. Thayer (1907) in his article on "Malaria" in Allbutt and Rolleston's "System of Medicine," having divided relapses into those at short intervals, and those after long intervals, writes: "It is not impossible that these late relapses may be due to parthenogenetic segmentation of macrogametes, such as has been described by Schaudinn. We have seen appearances exactly similar to his." Minchin (1912) defines parthenogenesis as "the power to develop, without syngamy possessed, by a sexually differentiated gamete" which under normal circumstances could do so only after syngamy with a gamete of the opposite sex. To this it must be added that the gamete which has this power is always the female.

Although parthenogenesis is said to be common among metazoa it would appear that the chief evidence of its occurrence among protozoa is the evidence adduced by Schaudinn in the case of P. vivax which we are to examine. We cannot therefore look for general evidence in support of the special case. But the special case in this instance, even if Schaudinn's interpretations of his figures be right, is not an example of parthenogenesis as defined above. Strictly speaking the term parthenogenesis would apply only if the female gamete without syngamy went through its exogenous cycle and not if it reverts to a schizont and goes through the endogenous cycle. The latter may still be true, but would not be evidence of parthenogenesis among protozoa.

Coming now to an examination of Schaudinn's original figures as reproduced in colour, I must refer the reader to the Plates that accompany his work on P. vivax (1902). But before examining his figures in detail I wish to point out that those he interprets as reversion (Rückbildung) and schizogony of the female sporont (macrogamete) are all from preparations made within forty-eight hours before the actual paroxysm of a relapse three and a half months after the previous attack. To expect to find forms that initiate the series of schizogonous cycles of a relapse at that stage seems to be quite as unreasonable as to expect to find sporozoites on the twelfth day of the normal incubation period of a first attack. That he should have done so is all the more astonishing as he had been examining the blood of this particular patient for days before. During his earlier examinations he had found gametocytes only; then nearer to the actual paroxysm he found in addition to gametocytes a few young trophozoites (ring forms); and lastly, forty-eight hours before the paroxysm, he found these forms which he
pieced together, building up a structure to explain relapses, which, under the name of parthenogenesis, has enjoyed a reputation which to this day has never been seriously disputed.

Schaudinn recognized that among double infections of red cells one of the forms might be a schizont, and the other a sexual form. This he illustrates by figs. 90 to 93, Plate V, accompanying his work on P. vivax. Figs. 90 to 92 show growing forms, each form separate and distinct from the other, so that their identity could not possibly be mistaken. Fig. 93 from a film taken during the height of a paroxysm (the fifth of an attack) shows a schizont divided up into merozoites in combination with a practically full-grown male gametocyte. If this figure be compared with fig. 110, Plate VI, where schizogony has reached a similar stage, but which, according to Schaudinn, represents schizogony of a macrogamete, the only structural difference seems to be that in fig. 93, Plate V, the gametocyte is a male, while in fig. 110, Plate VI, it is a female. Text

Text Fig. 1—Being tracing of Schaudinn's fig. 93.

[All Schaudinn's figures were magnified about 2,250 times linear.]

fig. 1, a tracing of fig. 93, is introduced here so that it may be compared with text fig. 8, but as these text figures are introduced simply to facilitate description the reader is again referred to Schaudinn's original reproductions.

Believing then that Schaudinn misinterpreted his findings I will now endeavour to give a natural explanation of why gametocytes are the earliest forms to be found in relapses, while in normal first attacks they do not usually appear before two or three paroxysms have taken place; and to show how Schaudinn's figures may be interpreted quite naturally in accordance with established facts without the necessity of bringing forward a hypothesis, which, to say the least, would require a good deal of extra evidence to establish its probability.

Passing over relapses at short intervals because the early presence of gametocytes in such cases requires no special explanation, I may say that in every case of relapse after a long interval
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in which I have examined the blood during the first paroxysm of the relapse, I have found gametocytes, male as well as female, present in ordinary films of the peripheral blood. I found them also during the first paroxysm of a very long delayed first attack of malaria fever (about eight months after the patient had left a malarious district). Figs. 29 and 30 (Plate) show a male and a female gametocyte from this case. In the case referred to under Addendum, Part I, I found gametocytes along with schizonts, two days before the first slight febrile manifestations of a relapse of an unsuspected benign tertian infection occurred. Schaudinn, as mentioned above, found them earlier still, though well within the possible incubation period of the relapse, and to begin with they were the only forms of the parasite he found. It is not necessary, however, to imagine that gametocytes found at those early stages of a relapse must have survived the long healthy interval that may have elapsed since the former attack. In normal first attacks, gametocytes, it is said, are not usually found before two or three paroxysms have taken place. The reaction of the host against the parasite is said to stimulate the production of the propagative phases. This is the same as saying that provision is made for the continuation of the species when the environment begins to be unfavourable to the life of the individual; and following, as it does, a much wider general law, this explanation may be accepted as correct so far as it goes. But in the case of a relapse the conditions are reversed. Here it must be assumed that the environment from being less favourable is becoming more favourable to the individual, and therefore, apart altogether from the question of what may be the starting point of the fresh series of schizogonous cycles, it is to be expected that a large proportionate number of gametocytes would be produced during the earlier generations of the series, more especially if the change from unfavourable to favourable, whatever it may be, is gradual and prolonged. If quick and complete, it is conceivable that gametocytes would not be found at the beginning of a relapse any more than at the beginning of an ordinary first attack; but, as said above, gametocytes have been present during the first paroxysm of all the cases of relapse that I have so far examined.

Schaudinn's figures, which he interprets as stages in the schizogony of macrogametes, arrange themselves quite naturally into two groups.

The first group comprises figs. 104 to 106, and deals with changes in the nucleus of macrogametocytes, which Schaudinn
interprets as the preliminary stages in the schizogony. The second group comprises figs. 107 to 110, and deals with a much more complicated arrangement. Each figure is divisible into two portions, one portion containing a number of deeply staining nuclei, and the other portion containing a single feebly staining nucleus which Schaudinn says becomes more and more feebly staining, but which, in other respects at least, seems to be indistinguishable from the nucleus of an ordinary female gametocyte. All these figures were from preparations taken between 7 a.m. and 8.15 p.m. of the same day, from a single case within forty-eight hours of a febrile paroxysm. Presumably they are from preparations taken at different hours, and if any inference as to the feebly staining nucleus becoming more feebly staining is to be drawn, it should be
from comparing it with the nuclei of normal gametocytes on the same slide, and not from comparing the nuclear stain of a figure from one slide with that of one from another slide. Schaudinn does not seem to have controlled his findings in any way; as for example, to determine if the forms in group 1 have any relation whatever to the schizogonous cycle, and if those in group 2 are confined to cases of relapse after long interval; and as already pointed out, his interpretations of these forms takes no account of the series of cycles that had already taken place. As a consequence of this neglect, he has, I believe, entirely misinterpreted his figures, and has pieced together two quite independent phases of the parasite to form a scheme of development in support of his so-called parthenogenesis theory of relapses. With the aid of coloured drawings, 2 to 36 on Plate, I hope to show (a) that Schaudinn’s figs. 107 to 110 are to be interpreted as representing combination forms, each consisting of an ordinary female gametocyte (full-grown or nearly so), and a schizont undergoing schizogony which had occupied the same red cell, and are so closely interlocked that they appear to be fused into one; and (b) that figs. 104 to 106 are mature female gametocytes, exhibiting early stages of, or preliminary steps towards, maturation that have taken place during the interval between the time that the blood was shed and the time that the film was fixed, or had dried on the slide. (a) Beginning with the forms included in group 2—figs. 2 to 30 show: (1) that though these forms are found at the beginning of relapses after long intervals, they are not confined to such cases; (2) that along with these forms we find other two combinations, so that all three possible combinations of gametocyte and schizont are found in the same film or in films of the same blood taken at the same time, viz., “gametocyte + schizont,” “gametocyte + gametocyte,” “schizont + schizont”; (3) that by appropriate staining many of these compound forms are clearly seen to be combinations, and (4) that (2) and (3) are borne out by comparison with ordinary single schizonts and gametocytes in the same films. Thus Case 1, figs. 2 to 6 are from a relapse after an interval of six and three-quarter months. All are from the same film taken during the first paroxysm of the relapse. On the day but one previous to this the patient had felt chilly, and his temperature had been up for a few hours. Figs. 2 and 3 represent what on the so-called parthenogenesis theory would in this case be interpreted as schizogony of macrogametes. The pale staining nucleus in each of the figures is not paler than the average nucleus of single female gametocytes in the
same film. Fig. 4 shows the combination "schizont + schizont," and fig. 5 the combination "gametocyte + gametocyte." Fig. 6 from the same film shows an ordinary schizont divided into merozoites. Case 2, figs. 7 to 12 are all from one blood-film taken during the first paroxysm of a relapse in a case that had been having constantly recurring relapses, each lasting four or five days, at intervals of two weeks or thereby. Figs. 7, 8 and 9 show the combination "gametocyte + schizont" in which the dividing line between schizont and gametocyte can be fairly clearly traced. Figs. 10, 11 and 12 show the combinations "gametocyte + gametocyte" which as was to be expected were more numerous than the combination "schizont + schizont" in this case. Case 3, figs. 13 to 26 are all from one case. The patient was admitted to hospital on the ninth day of a relapse. He had had eight paroxysms, one daily before admission. Figs. 16, 17 and 23 are from a film taken during the rigor of the ninth paroxysm of the relapse, i.e., the fifth paroxysm caused by the series of generations to which they belong. All the others (13 to 15, 18, 19, 20 to 22, 24 to 26) are from another film three and a half hours earlier, or about three hours before this rigor began, and belong to the same generation as the last. In this case, and in contrast to the last mentioned, the combinations "schizont + schizont" were more numerous than "gametocyte + gametocyte." Figs. 13 to 23 show the three possible combinations very clearly, and the dividing lines can be traced in diminishing degrees of distinctness until the two forms of each of these combinations appear to be almost fused into one. Figs. 24 to 26 show different stages in schizogony of ordinary schizonts in the film from which figs. 13 to 15, 18, 19, 20 to 22 were drawn. Case 4, figs. 27 to 30, are from a blood-film taken during the first paroxysm of a first attack of malaria fever eight and a half months after the patient had left a malarious district. He had been in Salonika and neighbourhood for about eight months, and in the Struma Valley for two weeks before he was wounded. Up to the time he was wounded he had taken quinine every afternoon, but had not taken any since then. He was in Malta for about three months after he was wounded, and in the King George Hospital for nearly five months before the paroxysm referred to, the first he ever had, developed. Figs. 27 and 28 show the same combination forms of "gametocyte + schizont" (the latter, of course, dividing). In fig. 27 the gametocyte is a male, and in fig. 28 it is a female; while, arranged immediately below these, figs. 29 and 30 represent an
ordinary male and an ordinary female gametocyte from the same film.

Coming now to Group 1. It is a general rule that where vegetative and generative chromatin are combined in the same nucleus, the elimination of what is considered to be effete, or vegetative chromatin, is the first step towards maturation, and is a necessary preliminary to gamete formation. In the case we are considering this step naturally begins as soon as an efficient stimulus is given to the mature gametocyte when it leaves the vertebrate host. What constitutes an efficient stimulus is not definitely known, but it may be that a certain temperature and a certain amount of moisture, giving rise to a change in osmotic pressure, constitutes such a stimulus. Be this as it may, when once the stimulus is given to the mature gametocyte, the elimination is effected quickly, and the effete chromatin always stains more deeply than does the generative chromatin left in the nucleus. This is illustrated rather strikingly in the figures that illustrate “initial changes in the exogenous cycle of *P. falciparum*” dealt with in the next section of these notes. Figs. 31 to 36 show similar changes in the nucleus of gametocytes of *P. vivax*. All are from a slide prepared in the ordinary way with no conscious variation or arrangement of conditions different from those under which all the other slides dealing with this section were prepared. I have seen similar appearances in other slides, and they do not depend on the parasite being at any particular stage of its schizogonous cycle, but on there being mature gametocytes present in the blood when the films are made. The time in relation to the schizogonous cycle at which the film from which figs. 31 to 36 were drawn, excludes any chance of the nuclear change being the first stages in a schizogony. It was prepared at the height of a paroxysm from the same case and at the same time as the film from which figs. 7 to 12 were drawn. The separation of the dark staining effete or vegetative chromatin from the generative chromatin of the nucleus, and the earlier stages in its elimination, may be traced in figs. 31 to 33, while figs. 34 to 36 show it in the process of being thrown off. The “smaller stronger staining portion” in Schaudinn’s figures included in Group 1 may in the same way be interpreted, without having resort to any unproved hypothesis, as effete or vegetative chromatin that is being eliminated in the normal way, and that this elimination began after the blood was shed and continued during the interval up to the time that the film was fixed, or had dried on the slide.
SUMMARY, AND CONCLUSIONS FROM THE FOREGOING EXAMINATION OF SCHAUDINN'S PARThENOGENESIS VIEW OF RELAPSES.

(1) Schizogony of macrógametes as described by Schaudinn, even if it were established, cannot be regarded as evidence of the occurrence of parthenogenesis among protozoa. In the strict sense in which that term is used when dealing with metazoa, it could be applied in the case of *P. vivax* only if the female gamete without syngamy went through its exogenous cycle.

(2) Schaudinn's observations were not sufficiently controlled; and in interpreting his figures he seems to have taken no account of the incubation stage that precedes the febrile attack. All of his figures are from films taken within forty-eight hours before an actual paroxysm; and to expect to find the forms that initiate the series of schizogonous cycles that lead to a febrile relapse at that stage seems quite as unreasonable as to expect to find sporozoites on the twelfth day of the incubation stage of a first attack.

(3) The observations described in this paper show that compound forms such as Schaudinn figures are not confined to the particular class of case to which he assumed they were, that they are combinations of gametocyte and schizont, and further that the changes in the nucleus of gametocytes that he interprets as the earliest changes in his scheme of gameto-schizogony have no relation in time to the schizogonous cycle, but depend on the presence of mature gametocytes in the blood at the time the films are made.

(4) All his findings and figures can be explained and interpreted quite naturally in accordance with established facts without the necessity of resorting to unproved hypothesis.

(5) In building up a scheme in support of his so-called parthenogenesis theory to explain relapses, Schaudinn not only misinterpreted his figures, but he brought together two quite independent phases of the parasite.

Another view put forward to explain relapses assumes that schizogony goes on without intermission throughout the intervals between relapses. According to Minchin, "Ross believes that in the healthy intervals the number of parasites in the blood merely falls below that sufficient to produce febrile symptoms, and that a relapse is brought about simply by an increase in the number of parasites present." During the incubation period of an average first attack of malaria fever schizogony goes on regularly, and conditions being favourable only schizonts are produced, so that the
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parasite tends to multiply by geometrical progression, and very soon the numbers in the blood increase enormously. With P. vivax the average length of a normal incubation period is said to be about fourteen days, so that, beginning with the sporozoites inoculated, no more than about six cycles normally take place before the parasites that will complete their schizogony two days later are sufficiently numerous to cause the characteristic febrile reaction in the host. Ross's view (or the view ascribed to him by Minchin) explains perfectly a large group of cases that relapse at intervals approximating to the ordinary incubation period, and others where the interval has obviously been prolonged by the continuance of special conditions such as the exhibition of quinine. Other conditions not so obvious may also tend to lengthen the interval between relapses, and the production of gametocytes at the expense of schizonts would have this effect. But there are relapses that occur after long intervals (several months to a year or more) of apparently perfect health on the part of the host; and, after making every allowance, it is difficult in the absence of direct evidence of the activity of the parasite, or of its presence in the blood, to accept this view of uninterrupted schizogony to explain these cases. It is most probable, and I think it must now be assumed, that relapses are made possible by the survival in some way of asexual (not sexual) forms, but the way in which they survive is another question. It seems also reasonable to suppose that as long as these asexual forms remain in the blood of their host they must go on multiplying or die out altogether. But, it would not be contrary to what is known about protozoa in general, to suppose that the asexual forms that survive do so by finding their way out of the blood-stream when conditions become unfavourable to their existence there, and, adapting themselves to nutritive changes; enter a resting stage in their new environment. Speculations such as these can be useful only as working hypotheses. With better methods of blood examination the hypothesis that schizogony goes on without intermission throughout these long intervals of apparent health between relapses may be either proved or made more improbable. Meanwhile it seems more in accordance with present knowledge to assume that in these cases, and in cases of very long delayed first attacks, there is a resting state in which the asexual form of the parasite remains quiescent during the greater part of these intervals; that this stage is passed outside the blood-stream (possibly in endothelial cells of the spleen, bone-marrow, etc.); and that, re-entering the
blood-stream, the parasite becomes active again before the relapse for a time which corresponds roughly with the incubation period of first attacks, but which is liable to be considerably prolonged if the change from unfavourable to favourable conditions is gradual and prolonged, and so favours the production of a large proportionate number of gametocytes during the earlier schizogonous cycles. The occurrence of relapses in some treated cases where the relapse is due to multiple or to mixed infection seems to favour the idea of a protected quiescent stage of the parasite. In the case of mixed infections referred to above (p. 387), the parasite causing the relapse escaped the action of quinine in doses that it would not be expected to have withstood in the way it did if it had been in the blood-stream undergoing schizogony at the time that these doses were given.

(b) Initial stages in the sexual cycle of the parasite of malignant tertian—phases not previously figured or described (figs. 37 to 55, Plate).

The preparation from which figs. 39 to 43 and 46 to 55 were drawn was made under the following conditions: The most favourable temperature for the natural development of crescents in anopheles being from 28° C. to 30° C.—the slide on which the film was spread, and the moist chamber (a Petre dish with the bottom piece covered with moistened filter paper) in which it was placed (see below) were kept in an oven at 30° C. for some time before being used. From this they were carried to the ward wrapped in a towel at the same temperature. A moderately thick film of peripheral blood known to contain many crescents was spread on the slide without unnecessary delay, was breathed upon, and then placed in the moist chamber referred to. This was again wrapped in the towel and carried to the laboratory, and placed for a short time in the oven registering about 30° C. About ten minutes later the slide was taken from the moist chamber, and, while still wet, the film was exposed to the action of osmic acid vapour for fifteen seconds, in order to fix the parasites in the position in which they then were. The film was then allowed to dry, and as the last traces of visible moisture disappeared, methyl alcohol was poured over it, and was allowed to act for fifteen minutes. The excess of methyl alcohol was then shaken off, and the film was stained with Giemsa.

Figs. 37 and 38 represent typical male crescents, and figs. 44 and 45 female crescents from an ordinary blood-film prepared at the same time. Figs. 39 to 43 and 46 to 55 show the changes that took place in the preparation above described. From these figures,
it would appear that the limiting membrane of the mature female crescent is more resistant than that of the male. In the female this membrane appears to be more of the nature of a cyst wall, and from this the female gamete (maturation having already taken place) seems to escape. The opening, or rupture, through which the gamete escapes, is in the great majority of cases (ninety-eight per cent) situated at or near the middle of the concave aspect of the crescent. In the straight sausage forms it is also about the middle of the long axis. In the male traces of the limiting membrane may still be seen, but it appears to give way fairly evenly all over, with the result that the male now assumes a more or less spherical form. Shreds of ruptured membrane are sometimes to be seen clinging to the surface. Before exflagellation takes place, however, the male maturates in the same way as the female by giving off nuclear matter in the form of “polar bodies”—usually two—(figs. 40, 41, and 43), but often appearing as a single mass (figs. 39 and 42). These polar bodies stain more intensely than the gamete-nucleus, and are very conspicuous in the stained preparation. In the case of the male they are seen lying on the surface of the sphere (figs. 40 to 43). In the case of the female they may be seen in different positions as one mass or as two separate bodies. Thus in fig. 46 they are seen as one mass at the pole of the capsule nearest to the nucleus; in fig. 47 at the pole farthest from the nucleus. In figs. 54 and 55 they are seen as one mass also near one of the ends of the capsule, but projecting from the surface. In fig. 51 a single wedge-shaped mass is seen outside on the surface of the capsule near its middle opposite to the escaping gamete. In fig. 53 two distinct bodies are seen protruding from one end of the capsule. In figs. 54 and 49 the polar bodies are seen applied to the side of the protruding part of the gamete; in figs. 50 and 52 they seem to have been pushed out in front of it—as two bodies in fig. 50—and as a single mass in fig. 52. Figs. 46 to 55 show the gradual escape (or extrusion) of the gamete—first a gradually increasing quantity of body protoplasm, with a stream of pigment granules, then the nucleus, and lastly the remainder of the body protoplasm and pigment granules, leaving behind an empty capsule, with, it may be, the polar body on the surface (fig. 55).

Whether the above truly represents the natural development and the true sequence of events, or whether it is an artificial product, can only be conclusively proved by examination of a series of anopheles at short intervals after they have fed on crescent-containing blood. It is not enough to say that no such forms have
ever been observed in living coverslip and slide preparations such as are used to demonstrate the process of "exflagellation" of male gametes, for in that case the same argument of unnatural conditions would apply perhaps even more forcibly. The possibility of these appearances having been produced by drying may, I think, be dismissed at once. The plump appearance of the parasites, the different behaviour of males as compared with females, and the fact that the whole of the film was quite moist when it was exposed to the vapour of osmic acid which would fix the parasites in the position and form in which they then were, are all against such a possibility. The best examples of both male and female forms here figured and described are found in what appears to be medium thin parts of the film, but this appearance of moderate thinness in different parts of the dried film is very probably, in part at least, due to the proportion of moisture to blood cells that existed at these parts of the moist film. Change in osmotic pressure may be a more probable explanation. Miss Muriel Robertson (1911) suggested that in the development of fish trypanosomes in the leech the stimulus that initiated the developmental changes was the lowering of the osmotic pressure of the blood and the probable absorption of water by the trypanosome consequent upon this. In the same way the lowering of osmotic pressure in the blood in the case we are considering (and the same thing may happen in "anopheles") would supply the initial stimulus to developmental changes in the crescents; while the consequent absorption of moisture by the crescents would raise their internal pressure and bring about the further changes here depicted. The male, with its thin limiting membrane which gives way fairly uniformly all over, tends to assume the spherical form. But given the crescent (or curved sausage) form, and an uniformly unyielding membrane, as in the case of the female crescent, increase of internal pressure tending to straighten out the crescent would tend also to rupture the membrane about the middle of its concave aspect, and any weakness of the membrane at this part would determine the point of rupture. In this way the large proportion (ninety-eight per cent) of female crescents in which the point of escape is about the middle of the concave aspect may be accounted for. Owing to its consistence the naked gamete, or as much of it as had escaped, would naturally assume the spherical form.
REFERENCES


EXPLANATION OF PLATE.

[It will be noticed that the references are to coloured drawings, but owing to the difficulties in reproduction it has been found necessary to reproduce the plate in black and white.]

The magnification of all the figures is 1,500 times linear. All have been drawn and coloured by Miss Rhodes of the Lister Institute—camera lucida, an achromatic 8 mm. oil immersion lens, and incandescent gas-light being used. All are from preparations stained by the Romanowsky method. Figs. 1 to 36 are from films dried and fixed in methyl alcohol; figs. 37 to 55 from films fixed while still moist in osmic acid vapour, and then in methyl alcohol.

Fig. 1.—Normal red cell.

Figs. 2 to 6.—From a film of peripheral blood taken during the height of the first paroxysm of a relapse after six and three-quarter months. Figs. 2 and 3, which on Schaudinns's so-called parthenogenesis theory would be interpreted as schizogony of macrogametes, show the combination "gametocyte + schizont," the schizont undergoing schizogony. Fig. 4 shows the combination "schizont + schizont," both undergoing schizogony. Fig. 5 shows the combination "gametocyte + gametocyte." Fig. 6 shows merozoites resulting from the completed schizogony of a single schizont.

Figs. 7 to 12.—From a film of peripheral blood taken during the height of the first paroxysm of a relapse, after constantly recurring relapses at short intervals (two to three weeks). Figs. 7, 8 and 9 show the combination "gametocyte + schizont," the schizont undergoing schizogony, where the dividing line between gametocyte and schizont is fairly clear. Figs. 10, 11 and 12 show the combination "gametocyte + gametocyte." The remains of the red cell can be seen in figs. 10 and 11.

Figs. 13 to 26.—From films of peripheral blood taken on the ninth day of a relapse. The patient had had daily paroxysms for eight days preceding the day on which the films were taken. All the figures with the exception of figs. 16, 17 and 23 are from a film taken about three hours before the paroxysm began. Figs. 16, 17 and 23 are from a film taken during the "rigor" three and a half hours after the first film was taken. Figs. 13 to 17 show the combination "gametocyte + schizont." In figs. 13
To illustrate "Notes on Malaria," by John D. Thomson, A.M., M.B., C.M.
to 15 schizogony is fairly well advanced, in figs. 16 and 17 it is nearly completed. The dividing line between gametocyte and schizont is clearly seen in figs 13 and 14, but is much less distinct in figs. 15, 16 and 17. The remains of the red cell can be traced in fig. 14. Figs. 18 and 19 show the combination "gametocyte + gametocyte." The dividing line between the gametocyte and the remains of the red cell can be clearly seen in fig. 18, and are less distinct in fig. 19. Figs. 20 to 23 show the combination "schizont + schizont." Schizogony is fairly well advanced in figs. 20 to 22, and in fig. 23 it is nearly completed. The dividing line between the two schizonts and the remains of the red cell are fairly distinct in figs. 20 and 21. In figs. 22 and 23 no remains of red cell are to be seen, and there is no distinct line of division between the schizonts, but in fig. 22 schizogony has advanced slightly further in one schizont than the other. Figs. 24 to 26 show ordinary schizonts at different stages of schizogony with the remains of their red cells.

Figs. 27 to 30.—From a film of peripheral blood taken during the height of the first paroxysm of a first attack of malaria fever eight and a half months after the patient had left a malarious district. Figs. 27 and 28 show the combination "gametocyte + schizont," the schizont undergoing schizogony. No remains of red cells are to be seen. In fig. 27 the gametocyte is a male. In fig. 28 the gametocyte is a female. Fig. 29 is an ordinary male gametocyte, and fig. 30 an ordinary female gametocyte, and in each case the remains of the red cell are easily seen.

Figs. 31 to 36 show stages in the elimination of effete or vegetative chromatin from the nucleus of ripe gametocytes, which must have taken place during the interval between the time that the blood was shed and the time that the film dried on the slide. The remains of the red cells are clearly seen. Fig. 31 shows the separation of the deeply staining effete or vegetative chromatin from the pale staining generative chromatin in the nucleus. Figs. 32 and 33 show the smaller deeply staining mass separated from the nucleus, while figs. 34 to 36 show it on the surface of the parasite on being extruded.

Figs. 37 and 38.—Male "crescents" from an ordinary blood-film fixed in osmic acid vapour.

Figs. 39 to 43.—Maturation of male crescents. Fig. 39, early stage—body still elongated, "polar body," deeply staining at one end close to surface but still inside. In figs. 40 to 43 the parasites have become spherical, and the polar bodies are seen lying on or projecting from the surface. In fig. 40 the two polar bodies are distinct but joined together. In figs. 41 and 43 they are separate, and at some distance from each other. In fig. 42 they appear as one, or as one on top of the other. In all the nucleus is large and diffuse, and, though it stains readily, the polar bodies stain more deeply and are very conspicuous.

Figs. 44 and 45.—Female "crescents" from the same film as figs. 37 and 38.

Figs. 46 to 55.—Maturation of female crescents, from the same film as figs. 39 to 43. Here the limiting membrane retains its shape, and the gamete escapes from a rent or opening near the middle of the long axis, leaving behind or carrying with it the polar bodies. These bodies stain much more deeply than the nuclei, and are prominent features in the stained preparations. In fig. 47, a comparatively early stage, the polar bodies appear to be still within the capsule. In fig. 46 partly in and partly out. In all the others they are wholly outside. The gradual escape of the gamete from the limiting membrane, or capsule, can be traced from fig. 46 to fig. 55. In figs. 46 and 47 only part of the cytoplasm carrying a few of the pigment granules has escaped, and in each the nucleus is still some way from the opening. In figs. 48 to 50 the nuclei are nearer to the openings of escape. In fig. 51 the nucleus is half way out, still further out in fig. 52, fully out in figs. 53 and 54, although some cytoplasm and pigment granules still remain, and in fig. 55 the whole gamete has escaped, leaving behind an empty capsule, with in this case the polar body clinging to its surface.