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Differential Diagnosis of Bastianellii and Vivax Malaria

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With a view to establishing criteria—applicable, if possible, in field work—for differentiating infection with Plasmodium bastianellii *(of simian origin) from that with* P. vivax *(which is not infectious to* rhesus *monkeys), the authors describe the morphological characters of* P. bastianellii *as seen in simian and human blood and have followed the course of infection in these hosts.*

Human infections with P. bastianellii *were characterized by relatively severe symptoms associated with very low parasitaemia. While no absolute criteria for differentiation between these two plasmodia have been found, the authors suggest that the presence of a scanty infection in the blood associated with small, poorly formed schizonts, which do not fill the erythrocyte, and with trophozoites of unsubstantial form is to be regarded as possibly denoting a simian origin of the infection. Subinoculation of the blood into a* rhesus *monkey may be the only certain guide to identification.*

A subspecies, *bastianellii*, of *Plasmodium cynomolgi* was described by Garnham (1959) on account of certain constant and striking differences in the morphology and behaviour of a new strain, which had originated in a *Macaca irus* monkey captured in Pahang, Malaya. Further support for the differentiation of the parasite was dramatically provided (Eyles et al., 1960) by the sudden eruption of accidentally transmitted cases of the infection to men in laboratories in the USA, where the strain had been taken the previous year. It has been pointed out (*Lancet*, 1960), that the next stage of the research should be to discover how many of the local inhabitants of Malaya and the surrounding countries are infected with the parasite, but in order to be able to detect such infections, it is essential to have suitable diagnostic criteria, and, if possible, these should be applicable in the field. The parasite belongs to the " benign tertian malaria " group and the practical issue is how to differentiate it from *P. vivax*. The latter is not infectious to *rhesus* monkeys, and this character provides an absolute indication of the species involved, but it would be difficult to use this criterion on a large scale or in the field.

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We thought, therefore, that *Plasmodium bastianellii* in blood films of monkeys and man should be compared with *P. vivax*, and that the course of infection in the different hosts should be studied. This information was obtained for infections in the natural host (*M. irus*), in *M. mulatta* and in man.

P. BASTIANELLII IN MONKEYS

The behaviour of the parasite was observed in two *irus* monkeys and in many *rhesus*, usually after sporozoite infection, and its morphology was studied in wet and dry fixed films stained with Giemsa's stain.

The youngest ring forms (Fig. 1, 8 and 16) consist of a prominent nucleus lying alongside a portion of cytoplasm, with the vacuole often inconspicuous. In the earliest forms, no enlargement of the erythrocyte is seen and Schüffner's dots are inapparent, but as soon as the parasite has reached a certain size (Fig. 2, 9 and 17), these features quickly appear, and considerable enlargement and stippling become evident. The cytoplasm increases in amount and projects in irregular pseudopodia from the vacuole (Fig. 10), though the parasite is not excessively amoeboid; it occupies at least half the cell. Tiny grains of light brown pigment collect in an isolated patch at the extremity of the cytoplasm, and now the corpuscle is more enlarged, measuring as much as 12 μ instead of 7.5 μ in diameter, while Schüffner's

dots are coarse. The nucleus does not expand in size at the same rate as the cytoplasm, and it is not much larger in the late trophozoite than in the early ring form; good staining reveals the presence of a darker staining dot, or karyosome, in a lighter matrix (Fig. 10). A double nucleus or an accessory dot is sometimes seen (Fig. 2); multiple infections occur with up to five parasites per corpuscle.

As growth of the trophozoite continues, the vacuole disappears and the nucleus divides (Fig. 3). Schizogony proceeds in the usual way (Fig. 19), but there is a tendency for the nuclei to collect on one side of the parasite and the merozoites to project out in a fan-shape, leaving unused a fair amount of cytoplasm (Fig. 4). The pigment meanwhile has increased in amount and agglomerates in two or three lumps near the centre of the parasite. Eventually 14-20 merozoites are produced in the mature schizont after a cycle of 48 hours (Fig. 5 and 12); the usual number of merozoites is 16, but exceptionally figures above or below these limits may be obtained, especially at the time of crisis. Curiously enough the schizont often gives the impression of a smaller object than the trophozoite; the red cell may become battered and shrunk and the parasite itself seems to contract as it loses its vacuole.

Male and female gametocytes are produced; the immature stages are non-vacuolated, except for an unstained portion adjacent to the nucleus, which may persist until the gametocyte is three-quarters grown. The microgametocyte has the usual pinkish cytoplasm, interspersed with fine brown pigment; its nucleus is fairly large and consists of a lighter outer and denser inner portion (Fig. 6, 13 and 21). The macrogametocyte has blue-staining cytoplasm with fine pigment granules and a more compact nucleus (Fig. 7 and 14). The vacuolated zone near the nucleus in the younger stages may persist even when the parasite is mature (Fig. 22).

The course of the infection and the appearance of the parasite are much the same in *irus* and *rhesus* monkeys, though the parasitaemia, instead of reaching a maximum of about 1% of the erythrocytes—as in the former—may in the latter rise to 10%.

Infections of *P. bastianellii* in monkeys derived from either sporozoites or blood forms follow a consistent pattern. The prepatent period after infection with sporozoites ends after seven days with the initiation of parasitaemia. The parasites rise to a peak in about seven to nine days. When a crisis occurs, their numbers then sink to a low level, to be followed by a recrudescence in about two weeks' time. A strong immunity develops, which, however, fluctuates in intensity so that relapses may occur in the periods of susceptibility, but the infection dies out as a rule in one to two years.

P. BASTIANELLII IN MAN

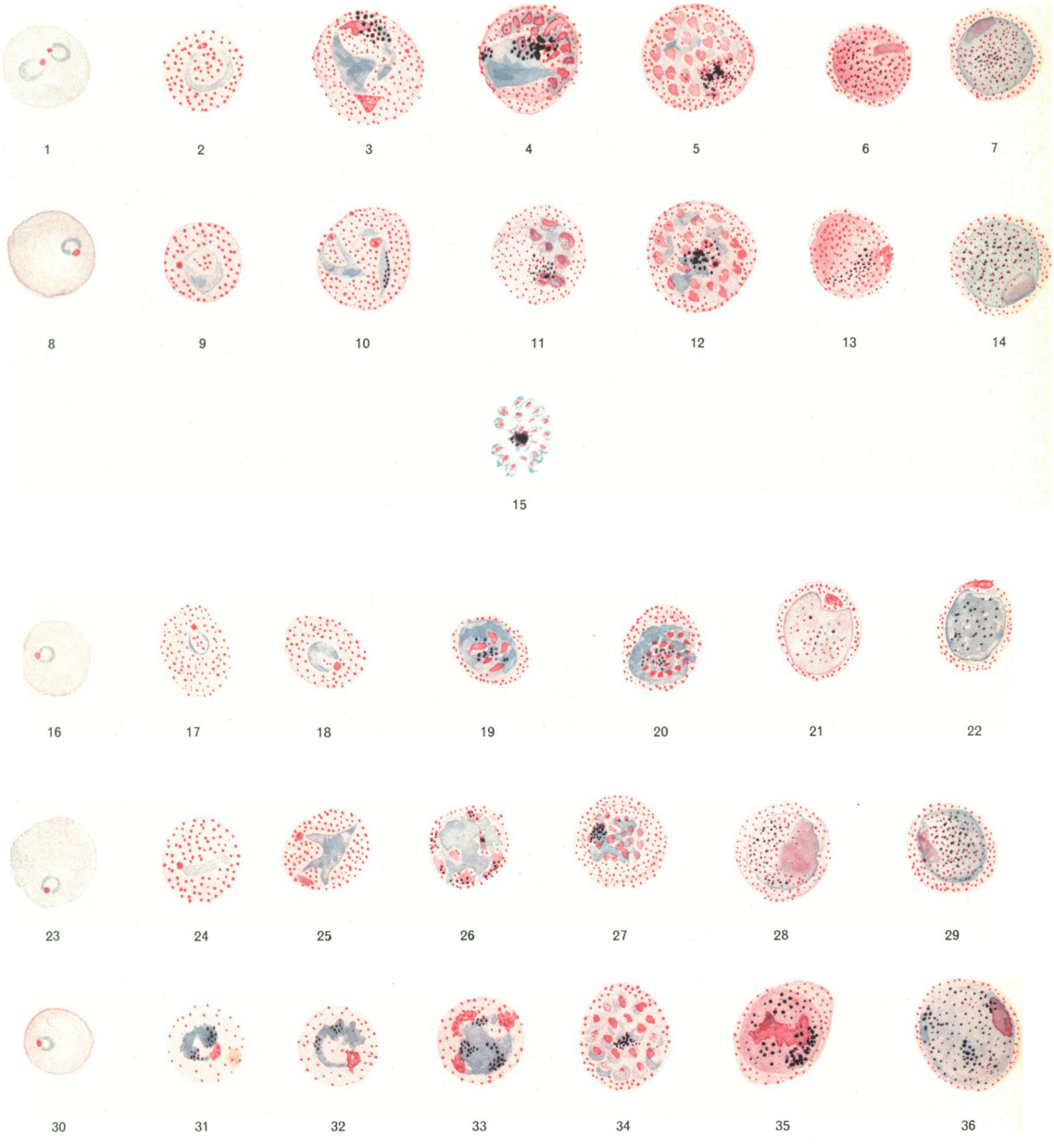
Human infections were observed in two laboratory workers accidentally infected and in twelve volunteers. Parasitaemia is always low in man and so it is difficult to obtain enough material for comparative purposes, but prolonged examination of all the infections eventually disclosed every stage of the parasite.

The early rings occupy about one-fifth of the diameter of the erythrocyte (Fig. 23). The nucleus is round or oval and forms part of the ring. As the parasite grows it throws out pseudopodia (Fig. 24 and 25); the erythrocyte enlarges, and becomes pale and stippled with Schüffner's dots. The pigment is relatively inconspicuous. The chromatin increases in amount and a lighter and darker portion may be evident (Fig. 25). The vacuole next disappears, the nucleus divides and the nuclei tends to become detached from the cytoplasm (Fig. 26). The immature schizont has a ragged outline, and when mature usually occupies only one-half to two-thirds of the host cell (Fig. 27). Up to 18 merozoites have been counted. The gametocytes are of the usual type, but tend to be smaller than those occurring in monkeys and do not entirely fill the cell (Fig. 28 and 29).

The laboratory infections occurred in two workers who had handled infected mosquitos on 1 November 1960. One became ill 11 days later with fever, headache and shivering, and tiny ring forms were found in his blood the following day. The other developed sweats and fever 17 days later, and paroxysms followed with tertian periodicity until 23 November, when immature schizonts were found in his blood for the first time. It took several days for chloroquine to bring about an abatement of the symptoms.

Twelve volunteers were infected with *P. bastianellii*: six by the bites of infected mosquitos (10-20 mosquitos per patient), and six by the intravenous inoculation of sporozoite suspensions from dissected salivary glands (five glands per patient). It was impossible to follow the whole course of infection in these two groups of patients, but they all developed malaria, as indicated below.

PLASMODIUM BASTIANELLII AND *PLASMODIUM VIVAX* IN SIMIAN AND HUMAN ERYTHROCYTES, STAINED BY GIEMSA'S STAIN [a]



[a] Fig. 15 is Bouin-fixed film, the remainder dry.

Fig. 1–15 : *P. bastianellii* in *Macaca mulatta*.
Fig. 16–22 : *P. bastianellii* in *Macaca irus*.
Fig. 23–29 : *P. bastianellii* in man.
Fig. 30–36 : *P. vivax* in man.

Group infected by bites

No. 1. Fever started on the 13th day after infection with a maximum temperature of 39.1°C (102.4° F) the following day. No parasites were found in either thin or thick films and the attack was cured with chloroquine.

No. 2. Fever started on the 12th day (38.9°C, or 102°F). On the following day one parasite was found in a thick film after a prolonged search. The attack was treated with chloroquine, but fever persisted for three days.

No. 3. Fever started on the 10th day (38.9°C, or 102°F). One parasite was found in a thick film on the 11th and 12th days, when the attack was terminated with chloroquine.

No. 4. The patient had a temperature of 37.8°C (100°F) on the 14th day after infection. No parasites were found and the attack was terminated immediately with chloroquine.

No. 5. Fever started on the 14th day (37.2°C, or 99°F), and one parasite was found in a thick film. The attack was terminated by chloroquine. On the eighth day, 5 ml of blood from this patient were inoculated into a *rhesus* monkey. *P. bastianellii* parasites were found 14 days later.

No. 6. Fever started on the 14th day (40°C, or 104°F) and one parasite was found in a thick film. The attack was terminated with chloroquine.

Group infected by intravenous injection of sporozoites

No. 7. Fever started on the 13th day after infection (37.2°C, or 99°F). No parasites were found and the attack was terminated with chloroquine.

No. 8. Fever started on the 13th day after infection (37.2°C, or 99°F). No parasites were found and the attack was terminated with chloroquine.

No. 9. Fever started on the 14th day (37.2°C, or 99°F). No parasites were found and the attack was terminated with chloroquine.

No. 10. Fever started on the 12th day (37.2°C, or 99°F). One parasite was found in a thick film and the attack was terminated with chloroquine. On the eighth day 5 ml of blood from this patient were inoculated into a *rhesus* monkey, which failed to become infected.

No. 11. Fever started on the 14th day (38.9°C, or 102°F). One parasite was found in a thick film. The attack was terminated with chloroquine.

No. 12. Fever started on the 11th day (38.4°C, or 101.2°F), and daily paroxysms occurred for the next nine days with a maximum temperature of 39.1°C (102.4°F). Parasites were first detected on the 13th day (48 hours after the onset of fever), and they became more numerous from the 15th day. The attack was terminated with chloroquine.

Like the other cases in man, these also showed the typical behaviour of *P. bastianellii* malaria, i.e., relatively severe symptoms with a low parasitaemia. These patients are of interest because probably all contracted the disease, with an average incubation period of 13 days in both groups. The prepatent period, as determined by inoculation of blood into a monkey, was at least as short as eight days, while parasites became demonstrable in thick films as early as the 11th day.

P. VIVAX IN MAN

A complete account of *P. vivax* as seen in Malaya (of particular relevance, because *P. bastianellii* originated in that country) was given by Field & Shute (1956). The morphology and type of infection produced by *P. vivax* are too well known to need a separate description here, but are discussed below in regard to differential diagnosis (see Fig. 30-36).

DISCUSSION

The human cases of *P. bastianellii* malaria described in this paper resemble those investigated much more fully by the American workers (Beye et al., 1961) and by Schneider (1961) in Paris. These workers showed that the parasite density was very low, never reaching a maximum of more than 0.01% of erythrocytes, often after a prepatent period of from three to four weeks, and with a duration of never more than 45 days. The human infections are characterized by the association of relative severity of symptoms with very low parasitaemia. This is the first important clue in differential diagnosis. Next *P. vivax* is a larger parasite with more substantial cytoplasm in the trophozoite stage (Fig. 31 and 32), while the mature schizont is a large mulberry form occupying most of the much enlarged erythrocyte (Fig. 34); the merozoites are round or ovoid instead of being definitely oval (Fig. 15). *P. bastianellii* in man has a cytoplasm of lighter texture; it is smaller at all stages of development; and the mature schizont

is of less regular outline and occupies only one-half to two-thirds of the host cell. None of these characters as presented by the blood picture is an absolute guide to identification; still less are minor features, such as colour of pigment, degree of amoeboid shape and intensity of stippling, which are so dependent upon the employment of identical techniques of staining or preparation of the blood film.

We cannot be more explicit than to state that in an enzootic region, the presence in a blood film of scanty parasites comprising small, rather ragged schizonts in stippled red blood cells would suggest the need for further investigation, in particular by subinoculation of the blood into a *rhesus* monkey.

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Since this paper was written, a good account of human infection with bastianellii malaria, as seen in American volunteers, has been published (Contacos et al., 1962). Interhuman transmission by mosquito bites was effected on several occasions. The British " variety " of the parasite appears to be more virulent in that the prepatent and incubation periods are much shorter than those reported in the American cases.

RÉSUMÉ

L'infection expérimentale humaine par *Plasmodium cynomolgi bastianellii*, agent du paludisme chez *Macaca irus*, étant facile à réaliser, on pouvait penser que cette infection pourrait se transmettre naturellement à l'homme. En d'autres termes, cette forme de paludisme serait une zoonose. Le parasite appartient au groupe *vivax* et il est fort difficile de poser un diagnostic différentiel entre *P. vivax* et *P. c. bastianellii* leurs caractères morphologiques étant très semblables au cours de la période sanguine de leur cycle.

L'étude de *P. c. bastianellii* a été faite au cours de deux infections accidentelles au laboratoire et d'infections expérimentales chez douze volontaires et chez le singe.

Chez l'homme la grande différence entre les deux agents est le degré de parasitémie; alors que *P. vivax* existe en nombre normal, *P. c. bastianellii* est toujours extrêmement rare ou même occulte, malgré la gravité de la maladie (la température atteignant 40°C ou plus). La période d'incubation chez l'homme est de 10-14 jours, bien que la période de prépatence soit de 8 jours comme on peut le démontrer par l'inoculation au singe.

Chez le singe, *M. rhesus* ou *M. irus*, *P. c. bastianellii* présente l'aspect typique d'une sous-espèce, c'est-à-dire qu'il est presque impossible à distinguer de l'espèce-type au cours du cycle sanguin. Le schizonte mûr n'occupe pas toujours l'érythrocyte entier, une masse résiduelle peut apparaître et la vacuolisation du cytoplasme est fréquente. Chez l'hôte naturel, la parasitémie est plus faible que chez le rhésus, mais beaucoup plus élevée que chez l'homme.

P. c. bastianellii, chez l'homme, diffère de *P. vivax* par sa morphologie: les schizontes jeunes ont une limite irrégulière et n'occupent, même à leur maturité, que la moitié ou les deux tiers de l'élément, les gamétocytes sont également plus petits et ne remplissent pas la cellule. De manière générale, le parasite est plus petit et naturellement plus rare dans le sang que *P. vivax*.

De tels critères ne peuvent suffire pour identifier l'agent infectieux chez l'homme dans les régions de zoonose. Cependant, la présence de symptômes d'une gravité hors de proportion avec la parasitémie et l'observation de parasites malformés du type vivax permettront de suspecter une infection à *P. c. bastianellii*. L'identification définitive peut être faite en inoculant du sang du malade à des singes rhésus: ceux-ci seraient atteints de paludisme typique à *P. c. bastianellii* en une ou deux semaines, mais ne réagiraient pas s'il s'agissait de *P. vivax*. Ce procédé, bien que fort facile à employer, n'est pas utilisable sur le terrain; on devra alors se contenter du diagnostic approximatif différentiel que permet le microscope.

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