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CONSIDERATIONS ON THE RELATIONSHIP BETWEEN
EXOERYTHROCYTIC FORMS AND RELAPSE IN MALARIA¹

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The question of the relationship between malaria relapse and the presence of exoerythrocytic forms (EE forms) has given rise to much discussion. For many years after the discovery of malaria parasites it was agreed that clinical or parasitic relapses (recrudescences, Ed.) were a consequence of the persistence in the blood of parasites which had survived the action of quinine or - later on - of mepacrine.

After the exoerythrocytic stage (EE stage) had been discovered and after it was found that schizontocides which destroy the blood parasites do not have the same effect on the EE forms, most authors who studied the problem of malaria relapse agreed that the EE forms were the cause of it.

However, some authors (Corradetti & Verolini, 1950; Ascoli & D'Alessandro, 1951) support Bignami's (1902, 1931) theory that relapses are caused by the persistence of parasites in the blood. It must be mentioned that before the discovery of the exoerythrocytic forms the explanation attributed to Bignami was the most reasonable one and corresponded to the general view.

After the discovery of the EE stage, Corradetti in particular opposed the view accepted by almost all malariologists, who considered the EE forms to be the cause of relapse. Many papers have been published on the subject and it is not possible to mention and discuss them all. Moreover, most of them concern avian malaria which is

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caused by a much larger number of parasitic species than human malaria. Since every plasmodial species has its own characteristic behaviour, as concerns both the EE stage and the erythrocytic stage, it is best to restrict the present discussion of the problem of relapse to findings in human malaria and in P. cynomolgi infection in monkeys, which is fairly close to vivax infection in man.

According to Corradetti & Verolini (1950, 1954) and Corradetti (1955), P. cynomolgi infection in monkeys and P. malariae infection in man produced relapses after experimental inoculation of infected blood, although no EE forms are to be found in the liver.

It should be pointed out that the infected monkeys were not given any treatment with antimalarials; recovery from the primary attack was spontaneous. Under these conditions there can be no doubt that the relapses occurring in three of the five monkeys on which splenectomy was performed were due to the persistence of a few rare parasites in the blood. Thus, the spontaneous cure observed was a clinical one.

The relationship between the presence of EE forms and relapse was deduced from findings in experimental P. vivax infection; after treatment of the primary attack relapses are very frequent if the infection has been induced by infected anopheles, whereas they are hardly ever seen after injection of infected blood. However, if the primary attack is not treated or if it is treated with inadequate doses of antimalarials, then relapses also occur in infections caused by inoculation of blood.

There can be no doubt that the relapses observed by Corradetti in P. cynomolgi infection of cured and splenectomized monkeys were due to the persistence of erythrocytic parasites in the blood, since it is probable that the behaviour of P. cynomolgi is the same as that of P. vivax. Nevertheless, Corradetti (1955) came to the conclusion that possibly the erythrocytic forms have a much greater chance of prolonged resistance to the action of immunity acquired by the host than do the EE forms; moreover, from his work on the EE stage in malaria in birds, monkeys and man, he maintains that there is no relationship between the EE forms and relapse.

It must nevertheless be remarked that the opinion of Corradetti and his co-workers is not shared by most authors who have studied the EE stage.

According to Bray (1957), the arguments in favour of the old theory - which regards relapses as the result of persistence of parasites in the blood - put forward by Corradetti & Verolini (1954) and Corradetti (1955) are largely vitiated by the fact that

they experimented on monkeys infected with P. cynomolgi by blood inoculation. Corradetti's experiments were, in fact, carried out under conditions which completely exclude the presence of EE forms in the experimental monkeys, for unlike what may happen in avian infection with P. gallinaceum and P. cathemerium, it is almost certain that the erythrocytic forms of P. cynomolgi and of human plasmodia do not give rise to EE forms. Although Corradetti's observations were correct, they do not prove that the EE forms are not the cause of relapse.

Everyone knows what is meant by "relapse" in the case of malaria, but there are various distinctions. Many authors have accepted the classification proposed by James, Nicol & Shute (1936), which classifies relapses of P. vivax infection into:

- (a) "recrudescences", which occur within eight weeks after the treatment of the primary attack with quinine and the disappearance of the parasites from the blood;
- (b) "relapses", which occur after a much longer period (normally between 8 and 24 weeks) during which the infection appears to be completely cured; and
- (c) "recurrences", occurring after 24 weeks.

James et al. (1936) considered recurrence "a striking event and why and how it happens is still a mystery", associated only with P. vivax infection and not observed in any other form of malaria. It is evidence that the authors did not wish to take into consideration P. malariae infection, in which the latent periods are much more prolonged than in benign tertian malaria. Their investigations dealt, in fact, solely with P. falciparum and P. vivax infections.

When the infection is induced by sporozoites, then according to most authors, the behaviour of the EE stage of human plasmodial species is as follows:

1. The EE stage of P. falciparum is very short; it is agreed that there is only a single generation of EE forms, which is derived from sporozoites (pre-erythrocytic forms or cryptozoites). All the merozoites produced by schizogony of the EE forms are haemotropic; consequently, in practice this is an infection involving almost exclusively the red cells, and, as established by James, Nicol & Shute (1936), only recrudescences occur, especially if the primary attack is treated with quinine which often does not kill all the parasites in the blood. However, if mepacrine is administered in a dose of 1 g on the first day and 0.30 g on the six following days, there is generally no recrudescence - the infection has been completely eliminated.

2. The EE stage of P. vivax often persists for a long time; the merozoites produced by the pre-erythrocytic forms are not all haemotropic; there are also histotropes. As surmised by Raffaele (1938) on comparing findings concerning the EE stage of P. elongatum, P. relictum, P. cathemerium and P. gallinaceum with what is seen in human malaria, it is now considered probable that the histotropic merozoites produced by the EE forms of human plasmodial species are generally very few in number. For observations on the EE stage of human plasmodia have been rather limited, revealing little apart from its existence. The researches of Bray (1957a, 1957b, 1958, 1960, 1963) on the EE stage of human plasmodia in the chimpanzee undoubtedly represent a remarkable advance, especially in our knowledge of the morphology of the EE forms, but do not help to elucidate their behaviour during the course of the disease.

The behaviour of the EE stage of P. vivax can be deduced from what is seen in P. cynomolgi infection in the monkey, where the erythrocytic parasites have morphological features similar to those of P. vivax. But perhaps this resemblance is not sufficient to conclude that the behaviour of the EE stage is the same in both species.

There are avian plasmodia such as P. relictum and P. cathemerium whose blood parasites are almost identical, whereas the EE stage develops in a quite different way. It is very brief in P. relictum, where the EE forms are rare and are found only at the beginning of the infection after the inoculation of sporozoites; they never invade the endothelium of the brain capillaries and apparently disappear after the parasites have commenced their development in the red cells. Personally, I have never seen them after subinoculation of infected blood. In P. cathemerium infection, the EE forms are very numerous, they invade the endothelium of the brain capillaries, they are found in large numbers after the infection has spread to the erythrocytes, even if it has been induced by inoculation of infected blood.

It is clear, therefore, that morphological resemblance between erythrocytic forms of different plasmodial species has no significance as concerns the EE stage which may develop in a quite different manner.

Moreover, the resemblance between P. cynomolgi and P. vivax is no more marked than that between their respective hosts and the course of the EE stage may not be the same.

It is probable that in human and monkey malaria, as happens in plasmodial infection of birds, the number of secondary EE forms (metacryptozoites) is variable. Experiments which have been carried out on monkey infection have almost always involved infection of these animals with an enormous number of sporozoites, a process which probably never occurs in naturally contracted malaria. In addition, the inoculation of large quantities of sporozoites seems to be an essential condition for observation of EE forms in the liver. Jeffery et al. (1952) had much difficulty in observing them after inoculation of 14 patients with P. falciparum sporozoites by mosquito bites and by intravenous inoculation; 8 of them were bitten by a number of infected anopheles varying from 379 to 1246 (on the average, 606 bites); 3 received hundreds of bites as well as being inoculated intravenously with hundreds of salivary glands; the remaining subjects received only the glands. The EE forms were observed in only one subject, who had received 8516 bites and 1403 infective glands.

It may be asked whether results obtained under these conditions can be of any use for throwing light on what happens in malaria infection contracted in nature. Massive inoculations of sporozoites give rise to a large number of EE forms and even if the histotropic merozoites produced by the EE schizonts are few in number, their total will produce a number of secondary EE forms sufficient to render them observable.

Shortt & Garnham (1948) examined 402 sections of liver from a monkey. They observed only two EE forms of P. cynomolgi 102 days after inoculation of sporozoites, precisely the day before a parasitic relapse. In the case of a monkey which on the seventh day of infection had shown eight EE schizonts in a liver section, Shortt, Bray & Cooper (1954) had to examine 80 sections of the same liver after 105 days to discover two EE forms.

These findings are of the greatest importance, since they show that the EE forms, although few in number, persist for a long time after inoculation of sporozoites. However, the origin of these few late EE forms has not been precisely established. According to Bray (1957) there are certain EE forms whose development is slower than that of the majority of the others. Perhaps he believes that these forms are responsible for relapses, since he does not seem too much in favour of the view that the development of a second generation of EE forms is possible.

Perhaps findings concerning the EE stage of P. cynomolgi are not sufficient to explain certain aspects of human malaria which are difficult to elucidate.

The duration of P. vivax and P. malariae infections is sometimes so long that it cannot be put down to the delayed development of EE forms. According to the classic schema of James, Nicol & Shute (1936), P. vivax (Madagascar strain) infection lasts only a year; according to Coatney & Cooper (1948), the length of infection with the St Elizabeth strain of P. vivax is about 15 to 16 months. However, it is known that benign tertian malaria can last still longer; a duration of two years was not uncommon in Italy when malaria was prevalent there, while Brumpt (1949) reports cases - although only an extremely small percentage - observed by Stannus (1930), Décourt (1931), Walton (1933) said to have lasted for 6, 8, 10 and even 33 years. But such cases - quite exceptional in P. vivax infection - are not exceptional in the case of quartan fever, where extremely long latent periods of up to 45 years have often been observed (Lentini & Tecce, 1955).

An attempt has been made to relate the phenomenon of the long latency of the infection to the presence of EE forms; according to Boyd (1953) it may be supposed that the development of some of the pre-erythrocytic schizonts stops and that they become latent, later resuming their development under certain conditions thanks to a mechanism which still remains to be determined. Griffith & Gordon (1952) believe that some sporozoites may reach the liver through the lymphatic channels much later than others.

In this connexion my opinion remains the same as I expressed at the Third International Malaria Congress, Amsterdam (Raffaele 1938), namely that the ratio between the number of histotropic and haemotropic merozoites, and consequently the number of secondary EE forms, is variable. I showed that this variability was particularly evident in infection of the chickens with P. gallinaceum. Trembly et al. (1950, 1951) succeeded in obtaining two strains of this parasite, one of which gave rise to almost exclusively exoerythrocytic infections, and the other to erythrocytic ones from which the EE forms were apparently absent. However, Lewert (1950) showed that the P. gallinaceum strains which cause infections of the exclusively exoerythrocytic type could be reconverted to the ordinary type of serial blood passages from one chicken to another.

The variability in the production of the two types of merozoite is not so evident in other species of plasmodia but it must be remembered that any variation in favour of histotropic merozoites increases the number of EE forms and consequently that of the haemotropic merozoites which they introduce into the blood on segmentation.

In theory, a single histotropic merozoite is sufficient to ensure that infection will persist in the body; in the case of human plasmodial infection I am still of the opinion I expressed some years ago (Raffaële 1938, 1940), i.e. that in schizogony of the EE forms of human plasmodia, haemotropic merozoites are much more numerous than histotropic ones; according to Fairley (1946), the EE forms of P. falciparum produce only haemotropes and there is only a single generation of EE forms.

In P. vivax and P. malariae infections, the production of histotropic merozoites is variable, although it is probable that in general it is less than that of the haemotropes. The number of secondary EE forms (metacryptozoites) is consequently very small, but it is calculated that each of these forms can give rise to 10 000 merozoites (Bray, 1957a), most of which are haemotropic and, in the absence of adequate immunization, they can recommence the erythrocytic stage and bring about relapse.

The variability of the ratio between the histotropic and haemotropic merozoites which I described (1938) in avian malaria and which I regarded as the cause of the difficulty in detecting EE forms in human plasmodial infections, was considered by Fairley (1949) and by Bray (1957a) as a possible explanation of the differences observed in the length of the latent period between the primary attack and relapse in P. vivax infection. According to Bray, during long latent periods the production of histotropic merozoites is higher than normal, and this ensures the persistence of the EE forms; when the ratio between the two types of merozoite returns to normal, the EE forms produce a large number of haemotropic merozoites, thus making relapse possible. Bray considers that the Madagascar and St Elizabeth P. vivax strains behave in this way, whereas the Chesson strain, which gives rise to relapse after a short period, produces EE forms and haemotropic merozoites more continuously, rendering reinvasion of the blood and consequent relapse more frequent.

At all events the part played by the EE forms in malaria infection doubtless explains (Raffaële, 1938) both the failure of causal prophylaxis employing schizontocides and the difficulty of preventing relapse in P. vivax and P. malariae infections.

Drugs which destroy the erythrocytic parasites have no effect either on the sporozoites or on the EE forms, which explains their persistence in the body after treatment. Acquired immunity seems to have no action on these forms: they were present in large numbers in the liver of an immune subject who, after being inoculated by Shortt, Garnham, Covell (1948) with P. vivax sporozoites, showed no fever or evident parasitaemia.

Although there can be no doubt regarding the relationship between the EE forms and malaria relapse, the frequent statement that the EE forms are the "cause" of relapse leads to misunderstandings. It would be better to say merely that they are the cause of persistence of the infection, for relapses are caused by blood parasites which may originate either from haemotropic merozoites produced by EE forms or from parasites persisting in the blood which have escaped the action of antimalarials. If this possibility is accepted in order to explain the recrudescence of P. falciparum infection, there would not seem to be any reason why it could not also be accepted for other types of infection.

However, a relationship between the EE forms and relapse cannot be rejected. It is proved by the failure of all preventive treatment and by the frequent negativity of the blood, even when tested by subinoculation, during the latent period.

None the less, just as there are asymptomatic parasite carriers who from time to time have attacks of fever, it must also be agreed that relapse can be the consequence of subpatent erythrocytic infection, but this is not a reason for denying the importance of the EE forms in the genesis of relapse.

Furthermore, neither EE forms nor latent parasitaemia explain the periodic return of symptoms which appear after several months of apparently perfect health. In P. malariae infection this period may stretch over decades. From this viewpoint, relapse still remains as much a mystery as it was for James.

RESUME

La question du rapport entre les rechutes du paludisme et la présence de formes exoérythrocytaires (formes E) a été l'objet de discussions. Pour bien des années, après la découverte des parasites du paludisme, on était d'accord de considérer les rechutes cliniques ou parasitaires comme conséquence de la persistance de parasites dans le sang, qui échappaient à l'action de la quinine ou - plus tard - de la mépacrine.

Quoiqu'il n'y ait pas de doute sur la relation entre les formes E et les rechutes du paludisme, affirmer que les formes E sont "la cause" des rechutes, comme on le fait souvent, se prête à des malentendus. Il vaudrait mieux se borner à dire qu'elles sont la cause de la persistance de l'infection. Car en effet, les rechutes sont causées par des parasites du sang, qui peuvent avoir pour origine soit des mérozoïtes hémotropes produits par les formes E, soit par persistance de parasites dans le sang, qui échappent à l'action des antimalariques. Si on admet cette possibilité pour expliquer les recrudescences de l'infection à P. falciparum, on ne voit pas pourquoi on ne pourrait pas l'admettre pour d'autres types d'infection.

Mais on ne peut pas refuser d'admettre la relation entre les formes E et les rechutes. Elle est prouvée par l'insuccès de tout traitement préventif et par la fréquente négativité du sang, même à l'épreuve de la subinoculation, pendant la latence.

Cependant, tout comme il y a des porteurs de parasites asymptomatiques qui, de temps en temps, ont des accès de fièvre, on doit admettre aussi que les récurrences peuvent être la conséquence d'une infection érythrocytaire sub-patente. Mais ce n'est pas une raison pour nier l'importance des formes E dans la genèse des rechutes.

D'ailleurs, ni les formes E, ni la parasitémie latente n'expliquent le retour périodique de manifestations morbides, qui arrivent après plusieurs mois d'apparente parfaite santé. Dans les infections à P. malariae, cette période peut se prolonger des dizaines d'années. De ce point de vue, la rechute reste encore un mystère, comme elle l'était pour James.

REFERENCES

- Ascoli, M. & D'Alessandro, G. (1951) Rif. med., 65, 1213
- Bignami, A. (1902, 1931) In: Marchiafava & Bignami, L'Infezione malarica, 1st and 2nd ed., Milan
- Boyd, J. K. S. (1953) Brit. med. J., 2, 392. Quoted by Bray (1957a)
- Boyd, M. F., ed. (1949) Malariology, Philadelphia, 2 vols.
- Bray, R. S. (1957a) Studies on the exoerythrocytic cycle in the genus Plasmodium, London
- Bray, R. S. (1957b) Amer. J. trop. Med. Hyg., 6, 514
- Bray, R. S. (1958) Amer. J. trop. Med. Hyg., 7, 20
- Bray, R. S. (1960) Amer. J. trop. Med. Hyg., 9, 455
- Bray, R. S. (1963) The Exoerythrocytic phase of malaria parasites. In: International Review of Tropical Medicine, 2, 41
- Brumpt, E. (1949) In: Boyd, M. F., ed., Malariology, 1, 102
- Coatney, G. R. & Cooper, W. C. (1948) Proceedings of the Fourth International Congresses on Tropical Medicine and Hygiene, vol. 1, 629
- Corradetti, A. (1955) Trans. roy. Soc. trop. Med. Hyg., 49, 311
- Coradetti, A. & Verolini, F. (1950) J. nat. Malar. Soc., 9, 327
- Corradetti, A. & Verolini, F. (1954) Riv. Parassit., 15, 65
- Décourt, P. (1931) Rev. Méd. Hyg. trop., 23, 32. Quoted by Brumpt (1949)
- Fairley, N. H. (1946) Trans. roy. Soc. trop. Med. Hyg., 40, 105
- Fairley, N. H. (1949) Brit. med. J., 2, 825
- Griffith, R. B. & Gordon, R. M. (1952) Amer. J. Trop. Med. Parasit., 46, 311
- James, S. P., Nicol, W. D. & Shute, P. G. (1936) Proc. roy. Soc. Med. Sect. trop. Med. Parasit., 29, 27
- Jeffery, G. M., Wolcott, G. B., Young, M. D. & Williams, D. C. (1952) Amer. J. trop. Med. Hyg., 1, 917
- Lentini, D. & Tecce, T. (1955) Riv. Malar., 34, 259

- Lewert, R. M. (1950) Amer. J. Hyg., 51, 155
- Raffaele, G. (1938) Acta Conv. Tertii de Malaria Morbis, Amsterdam, vol. 2, 545
- Raffaele, G. (1940) Riv. Malar., 19, 195
- Shortt, H. E., Bray, R. S. & Cooper, W. (1954) Trans. roy. Soc. trop. Med. Hyg., 48, 122
- Shortt, H. E., Garnham, P. C. C. (1948) Brit. med. J., 1, 1225
- Shortt, H. E., Garnham, P. C. C. & Covell, G. R. (1948) Brit. med. J., 1, 547
- Stannus, H. S. (1930) Trans. roy. Soc. trop. Med. Hyg., 24, 375. Quoted by Brumpt (1949)
- Trembley, H. L., Greenberg, J. & Coatney, G. R. (1950) J. nat. Malar. Soc., 9, 68
- Trembley, H. L., Greenberg, J. & Coatney, G. R. (1951) J. nat. Malar. Soc., 10, 76
- Walton, A. J. (1933) E. Afr. med. J., 9, 308. Quoted by Brumpt (1949)

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