PATHOLOGICAL STUDIES IN MALARIA

I. NOTES ON 192 ROUTINE AUTOPSIES PERFORMED IN SAO PAULO, BRAZIL, WITH SPECIAL REFERENCE TO PIGMENTATION AND SPLEEN SIZE

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This review or pathological findings with respect to malaria in a series of 192 autopsies performed in the Institute of Pathological Anatomy of the Faculty of Medicine of São Paulo is presented by way of introduction to a corresponding study in Porto Rico where during the past three years 163 autopsies have been similarly investigated. It should be stated that data herewith discussed formed the basis of an earlier paper on malarial pigmentation in the Revista Medicina of São Paulo, (1925, Vol. XII, p. 23), a local institutional publication.

The anatomical evidence of malaria, apart from the blood changes, consists essentially in the deposition of the characteristic pigment in certain organs and tissues. While in acutely fatal infections degenerative parenchymatous lesions in the viscera of varying degree may be observed, these are in general rather less marked than in many other acute febrile diseases. It is interesting also that the distinguishing anatomical feature, pigmentation, persists for a while at least, after eradication of the infectious agent, particularly if the infection has lasted for some time.

This persistence, which is due to the slow elimination of the pigment, makes possible the recognition at autopsy of recently cured as well as active or latent infections. We have; therefore, in the systematic study of autopsy material a valuable means of estimating the endemicity of malaria in any given region, provided, of course, that the cases coming to autopsy are fairly representative of the general population. In any case it may be maintained that wherever there is an active autopsy service the post mortem findings should constitute a valuable supplement to the knowledge gained by the two common methods of estimating endemicity, namely blood examination, giving the so-called "parasitic index", and spleen examination, for the "splenic index". It is hardly open to argument that the percentage of error in carefully studied autopsy material is generally lower than in blood and spleen examinations. The

weakness of the autopsy method lies in the fact that the cases are generally relatively few in number and often not truly representative of the population.

In the present study we have had in mind the question of the relationship of malarial pigmentation to the size of the spleen as well as that of endemnicity.

MALARIAL PIGMENT

As is well known, the pigment is formed within the parasite during its development and is set free at the end of schizogony or upon the death and disintegration of the sexual forms of the parasites. The black granules thus circulate freely in the blood until taken up by leucocytes or endothelium. There seems no doubt that the pigment is derived from the hemoglobin of the erythrocytes probably through the action of a ferment. The idea, long held that the pigment belonged to the group of true melanins is now generally discredited (Wells, Brown). Ascoli believes that while the pigment is not identical with any of the several well recognized derivatives of hemoglobin (hematin, hematoidin, hemosiderin), it is a compound in which the colored element is hematin. Brown, on the basis of careful chemical studies, goes further than this, maintaining that the malarial pigment is nothing more or less than hematin, and that the toxic phenomena of the infection, and in particular the paroxysms, are due to the action of this metabolic product.

Whatever may be the exact nature and rôle of the pigment it is sufficient for the present study to note that in respect to localization and morphology, there are certain characteristics which enable a morphologist of moderate experience to recognize it with little difficulty. The pigment is found in greatest abundance in the spleen, liver and bone marrow, though, particularly every organ and tissue. In the spleen, amorphous brownish black particles are seen, singly and in masses, intra-cellular, both within the sinuses and in the interstitial tissue. There is generally much less in the follicles. In chronic infections where the deposition is great the pigment tends to accumulate in amorphous masses in the pulp. In the liver, a similar deposition of the granules, singly or in masses, is seen. But here the localization is more clearly defined. The Kuppfer cells are practically the sole depository. Some authors, notably Ewing, have described malarial pigment in the hepatic cells, but we have not been able to confirm the observation. It is possible

that the author, as Brown has suggested, may have been confused by the presence of bile pigments and the precipitate produced by formalin to which reference is made in a subsequent paragraph.

Of the pigment deposition in other organs, nothing need be added, except to mention the striking accumulation in the cerebral capillaries in lethal cases, or occasionally, as Taliaferro and others have shown, in the placenta. (The "localization" phenomenon will be discussed in a later paper).

For the recognition of the pigment, there is a practical difficulty in the case of formalin-fixed tissues. Tissues remaining in this fixative for some days, frequently show deposits in vascular areas of a finely granular black pigment, morphologically much like that of malaria. According to Browicz, the pigment is probably hematin, derived from methemoglobin. With a little experience, it need not be confused with malarial pigment, since the formalin produced substance is diffusely scattered through vascular areas, and is not intra-cellular. In the blood-vessels, however, it may be found clumped on and about the leucocytes and endothelium, simulating phagocytosis. To avoid difficulty some other fixative than formalin should be used.

Regarding the fate of the pigment and the rate of its disappearance after the cure of the infection, very little is definitely known. Ziemann reported the absence of pigment in autopsies on negroes who were known to have been infected some years before death, but it is not clear from his statement that his observations included a histologic investigation of the organs. It is our experience that a relatively large amount of pigment is required to modify the gross appearance of either liver or spleen. The commonly described black or slaty organs have been the exception in our material.

Kelser and Kiener, failed to find pigment in the liver in a man who died "some months" after an attack, indicating the more rapid elimination of the pigment from this organ than from the spleen. It has been assumed that the pigment, in the various organs, after being taken up by phagocytes, is gradually broken down by intracellular ferments, and thus eliminated.

MATERIAL STUDIED

This consisted of tissues from 192 autopsies performed in the Institute of Pathological Anatomy in the four-year period, April 1921

to May 1925. During this time there was a total of 430 autopsies, from the majority of which, however, tissues appropriate for our study were not preserved.

In a majority of the cases the tissues were fixed in formalin. Zenker's fluid was the only other fixative employed. The sections were stained routinely with hematoxylin-eosin. Sections showing pigment of doubtful character were treated with hydrochloric acid and potassium ferrocyanide (Berlin blue reaction) to exclude hemosiderin. Solubility in strong alkaline solutions served to differentiate the pigment from carbon particles and true melanin.

The cases autopsied were largely adult males between twenty and fifty years old dying in the Santa Casa de Misericordia, the large general hospital of São Paulo. At least 75 per cent came from the interior of the state, where in many districts malaria is prevalent. Infections acquired in the city of São Paulo itself are said to be quite rare. As a matter of fact, only one of the 22 positive cases in our series was a resident of the city of São Paulo.

FINDINGS

Of the 192 cases, 22 were positive, that is, showed a deposition of malarial pigment in one or more organs, and 170 were negative. In 12 additional autopsies suspicious looking pigment was encountered but on account of the abundance of what was obviously formalin pigment, the cases were excluded from the series as being too doubtful to be classed as either positive or negative.

Of the 22 positives, 7 were examples of lethal infection, that is, the autopsy findings indicated that malaria was the only cause or one of two causes of death. (See Table 1.) In these, the pigment was found widely distributed, both extra and intra-vascular. The 5 cases in which sections of brain were preserved showed numerous pigment granules in the cerebral capillaries, in some instances completely occluding the lumen.

The remaining 15 positive were cases in which the pigment deposition was a subsidiary finding, the malarial infection being apparently incidental or cured and not constituting a definite factor in the death of the person.

Cause of death, age, sex, and weight of spleen of the positive cases are given in the two following tables, for lethal and subsidiary infections.

TABLE I

CASES OF LETHAL MALARIA

No.	Sex	Age	Weight of spleen gms.	Cause of death
1	Male	27	550	Malaria and dysentery
2	Male	67	375	Malaria
3	Male	47	550	Malaria
4	Male	21	350	Malaria and uncinariasis
5	Male	24	330	Malaria and acute suppurative peritonitis (origin undetermined)
6	Male	70	270	Malaria
7	Male	56	820	Malaria

TABLE II

CASES OF SUBSIDIARY MALARIA

No.	Sex	Age	Weight of spleen gms.	Cause of death
1	Male	30	170	Chronic pulmonary tuberculosis
2	Male	50	420	Aneurysm of aorta
3	Male	23	250	Chronic glomerulo-nephritis
4	Famale	18	160	Acute yellow atrophy of liver
5	Male	25	340	Sarcoma of testis
6	Male	23	100	Lobar pneumonia
7	Male	22	420	Lobar pneumonia
8	Male	45	100	Carcinoma of esophagus
9	Male	19	800	Suppurative arthritis: septicemia
10	Male	23	270	Typhoid Fever
11	Famale	30	500	Generalized blastomycosis
12	Male	26	150	Chronic pulmonary tuberculosis
13	Male	16	?	Pemphigus
14	Male	35	?	Syphilis of larynx and liver
15	Male	35	?	Beriberi

It is interesting to note that all the positive cases were adults and that only 2 of the 22 were women. The character of the autopsy service explains the predominance of adults and males. Very few autopsies are done here on children and the proportion of sexes among the available cadavers is approximately four males to one female.

Summarizing the findings it is seen that evidence of malarial pigmentation was found in 22 or 11.5 per cent of the cases. It may be pointed out that a clinical diagnosis of malaria was made in very few of the cases, which is not surprising in view of the fact that most of the patients were suffering from some more serious malady, and that examination of blood smears is not a routine procedure in the hospital. In view of the known endemicity of malaria in many parts of the interior of São Paulo, from which a large proportion of the patients came, the incidence among the autopsies is not higher than might be expected.

The tissues were not searched systematically for malaria parasites, with the object of differentiating between active infections and cured or latent cases, since in most instances suitable material for such studies was not available. It is possible that in a considerable proportion, especially in the cases now showing splenic enlargement, the infection may have been cured. This point will be referred to again in a later paragraph.

That the splenic enlargement regularly associated with acute malaria may disappear under appropriate treatment, is well recognized. In chronic cases of long duration with hard, greatly enlarged spleens, however, there may be some doubt as to whether the organ can become entirely normal again even if the infection be eliminated. A review of the findings in our cases suggests some comments on this point.

As would be expected, the spleen was markedly enlarged in all of the 7 cases of lethal malaria, the recorded weights in six of these being 270, 330, 375, 550 and 820 grams. In the seventh case, the measurements given indicate a weight of at least 350 grams. On the other hand, as Table II shows, the size of the organ varied greatly in the 15 cases, in which the malarial pigmentation was a subsidiary finding, that is, where the cause of death was some other disease. The average weight in this group was 307 grams, in comparison with 298 grams in the negative cases.

In the subsidiary group three spleens weighing more than 400 grams each were encountered. These excessive weights have little significance, however, since in each case the splenomegaly could be explained on the basis of chronic passive congestion or "acute splenic tumor", or, as in the case of blastomycosis, to infectious lesions in the organ itself.

The question may be raised, of course, as to what is the normal weight of the spleen. We know that this varies with both the size of the body and the age. Owing to its large content of lymphoid tissue, which tends to undergo atrophy with advancing years, the spleen is proportionately smaller in old people than in young adults. According to Gray, the relation to body weight is 1:350 for young adults, while in the aged the proportion may reach 1:700. If we take as the normal for an individual of 20–30 years, weighing say 60 kilos, a spleen weight of 150–160 grams, with gradually diminishing figures for the higher decades, it is seen that in spite of complicating diseases, which tend generally to cause splenic enlargement, one

spleen in these "subsidiary" malarial infections was approximately normal in size, and two definitely subnormal. The proportion of these undersized spleens in the malarial and non-malarial groups, it is interesting to note, was practically the same, as the following figures show:

Negative cases: 170; small spleens, 23; per cent 13.5.

Positive cases: 22; small spleens, 3; per cent 13.7.

The ages and weights of the small spleens in the non-malarial cases are shown in the following table:

TABLE III

NEGATIVE CASES WITH SMALL SPLEENS

No.	Age	Weight of Spleen
1	25	30
2	54	80
3	45	120
4	44	110
5	66	90
6	60	90
7	40	90
8	30	140
9	28	130
10	18	150
11	30	110
12	32	60
13	64	70
14	21	120
15	23	135
16	54	35
17	30	140
18	24	60
19	33	90
20	24	100
21	40	120
22	28	90

There are at least two possible explanations for the small pigmented malarial spleens:

1. That the cases represent cured infections, in which the organ has not only returned to normal but become reduced in size, owing, perhaps, to a slight diffuse fibrosis.

2. That there are cases in which the spleen was originally, that is, congenitally, abnormally small, and that therefore a slight reaction still left the organ smaller than "normal".

Whatever may be the interpretation, it would appear from a study of these small spleens that the presence of malarial pigment *per se* does not excite a definite, or at least a persistent vascular or cellular reaction sufficient to cause enlargement of the organ. In this connection it is significant that we failed to find any correlation between the size of the spleen and the amount of pigment present. In case 3 of Table II, for example, the spleen weighing only 250 grams, contained far more pigment than several others weighing twice as much. Daniels in a study of tropical splenomegaly in British Guiana many years ago called attention to the lack of correspondence between spleen size and pigment deposition. We are therefore inclined to the view that it is the malarial parasite itself or some non-pigmentary product which induces the congestion and the cellular accumulation with the consequent splenic enlargement, so commonly associated with active infection.

In any estimate of malarial endemnicity based on the "spleen rate", these cases with small spleens would, of course, fall in the normal group. A further study of such spleens should, therefore, be made to determine whether they are infective or whether the pigmentation is merely, as we suppose, a residual lesion, which may persist indefinitely.

SUMMARY

Malarial pigment, popularly but erroneously called melanin, is so characteristic in its physical properties and localization in the tissues, that it is possible to recognize at autopsy not only acute and chronic malarial infection, but also recently cured cases.

It is suggested, therefore, that the systematic search for pigment in all cases autopsied in malarious districts should yield valuable data respecting the endemnicity of the infection.

Such a study of 192 autopsies in São Paulo, Brazil, shows an incidence of malarial pigmentation of approximately 12 per cent. This figure might be designated the "pigment rate" or "autopsy index".

The 22 positive cases included 4 in which malaria was the only cause of death, and 3 in which it was considered one of two causes. In the remaining 15 cases the pigmentation was a subsidiary finding.

In this "subsidiary" group, great variation in the size of the spleen was noted, there being several which were normal or even subnormal in weight. This observation suggests that pigmetation *per se*, does not produce enlargment.

The observation further suggests that the pigmented malarial spleen in latent or cured infections may not enlarge as the result of passive congestion or bacterial infection to the same extent as the non-malarial organ, though the cases observed were too few to admit any conclusion being drawn.

(A complete bibliography will accompany the second paper of this series dealing with studies in Porto Rico).