THE TIME REQUIRED FOR THE EXAMINATION OF THICK BLOOD FILMS IN MALARIA STUDIES, AND THE USE OF POLYCHROMATOPHILIA AS AN INDEX OF ANEMIA.¹,²

BY

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In a recent survey of school children in a village (Yerondas) of Greek Macedonia, we examined the same 65 children on three dates: November 18, November 22, and December 23. At that time of year the temperature was low and there were few anophelines in the village, so there should have been no transmission of malaria during the experiment. All blood films were examined by the writer.

Accuracy of malaria parasite index obtainable in a single survey.

The percentages of children found parasite-positive were, in order of the dates of examination: 53.8, 50.8, and 50.8. Only 36.9 per cent were positive in all three examinations, and 33.8 per cent were negative in all. The species of parasite found in a child more than once positive was nearly always the same, but the degree of infestation (number of parasites per unit of blood) varied greatly. A minimum of 66.2 per cent of the children proved to be infected; that is, were positive in at least one of the three examinations.

It appears that a single examination can give no more than an approximation to the actual number infected; it is perhaps remarkable that our three examinations gave percentages so similar. Judging from our experience in previous tests of this kind, it is probable that the re-examination of negatives, continued monthly throughout the winter,

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² I am under obligations to Dr. H. Muench for working out the correlation shown in the tabulation on page 29, and to Dr. J. B. Rice for determining the hemoglobin index compared. Also to Dr. A. Mandekos for examining the spleens of children in the survey described in this paper. Dr. Fritz Weyer of the Institut für Schiffs-und-Tropenkrankheiten, Hamburg, Germany, was kind enough to look up the reference on the first use of the simplified thick-film method.
would bring the cumulative positive percentage of this group to 80 or
more, and this might far exceed the spleen index. The spleen index
was, in order of the dates of examination: 76.6, 79.7, and 75.4; the
average spleen was 1.78, 1.84, and 1.78 (examination by Dr. A.
Mandekos).

An approximation, then, to the malaria parasite index is all that we
can expect from a single survey. But such approximation is of great
value for the purposes for which malaria surveys are usually made:
namely, the measurement of the results of antimalarial work; com-
parison of the prevalence of the disease in different seasons, years, and
localities; relation of spleen and parasite indexes; determination of the
species of *Plasmodium*; degree of infestation; and the like. For all
comparisons, uniformity of method of examination is of course highly
desirable.

*Time necessary for examination of thick blood films.*

With regard to the time to be devoted to the examination of blood
films,—often considered a tedious and time-consuming work,—we
not only need uniformity but also should know the minimum of time
required to obtain a result suitable to the purposes of the survey. The
less time one gives to the examination of a preparation, the more
specimens he can examine; thus the error due to small numbers is
diminished. Fewer examiners are needed, and the task can be
assigned to those better skilled and more conscientious. Again, it is
often of importance to know the results of a survey as early as possible.

To prescribe a standard time for all examiners is open to the same
objections as to assign a standard time for reading a certain article in a
magazine; such a standard is useful only within very wide limits. One
person can learn as much in two seconds of examination of a microscopie
field as another could in a half-minute or more. So it is useful for each
examiner to determine for himself the relation of the amount of
examination (in terms of time or of microscopic fields) to the parasite
percentages obtainable.

In order to obtain a working formula for myself, I noted the amount
of examination required to find the first parasite in a series of 1232
thick films. At least 150 fields (1/12 oil immersion and 7 × ocular)
were devoted to negatives. At the end of the full examination, 150
fields, 34.0 per cent positive had been obtained; at the end of 125, 33.4
per cent positive; at the end of 100, 32.7 per cent; at the end of 75,
30.9 per cent; at the end of 50, 28.5 per cent; and at the end of 25, 25.3
per cent. It appeared that examination of 100 fields was as good as
examination of 150 for the purposes of our surveys; possibly examination of 75 would suffice. The error due to limiting the amount of search is really less than that shown by these percentages, since one prolongs the search if anything suspicious of parasitism is seen, such as pigment, chromatin-like bodies, high degree of polychromatophilia, or the like.

The error does not seem to be materially greater in groups with a much lower percentage positive than that shown in the preceding paragraph. I examined 244 specimens from four villages, in none of which the parasite index exceeded 10 per cent. At the end of the full examination, 150 fields, a percentage positive of 8.6 had been obtained; at the end of 100 fields, a percentage of 8.2. In another test, 90 specimens were selected, all known to be positive but with a very small number of parasites in each. Ninety-six per cent positive was obtained at the end of 125 fields and 90 per cent at the end of 100. Such a condition as that found in the last experiment, with many positives and all with very small numbers of parasites, is rarely or never seen in routine surveys. Usually the positives in a village exhibit greatly varying numbers of parasites; where the percentage positive is very small it may be due to a few persons, often chronic carriers, who have relatively large numbers of parasites.

In my case, the examination of 100 fields requires approximately 2 minutes. The time required for a number of specimens varies, of course, with the percentage of positives and with the time devoted to counting parasites, or searching for gametocytes, mixed infections, and the like. It is my routine to devote at least 50 fields to positives even though parasites may be found in the first field examined. On the average I examine 15 or 20 specimens an hour.

It is assumed in the foregoing discussion that one is examining well-prepared specimens. It is much easier to recognize parasites quickly when they are properly stained and occur among normal blood elements only, than to pick them out from among bacteria, yeasts, and various other kinds of dirt rubbed from the skin of the patient. The comparison with the printed page occurs again; it is difficult to read quickly a page of specked and soiled paper with faded or badly printed characters.

Technique of preparing thick and thin blood films.

For the preparation of thick films two rules may be emphasized:
(a) Avoid rubbing the skin of the patient with the slide; it is best to touch only the blood-drop even when the skin is presumably well cleaned. (b) Get plenty of blood for the thick drop. When, as is
rarely the case, one has to use only a small drop, it should not be spread widely, a maneuver which usually affords a preparation lacking the virtues of either the thick or the thin film.

The simplified method now in general use in preparing thick films consists of the simultaneous staining and dehemoglobinizing of dried unfixed films in diluted Giemsa stain. This modification was first used by the German expedition for the study of sleeping sickness in East Africa (1), (2), (3).

A type of thick film preparation which we find very useful for survey work is made as follows: The thick drop is placed near one end of a slide and a very small drop of blood placed just above it. This small drop is made into a thin film by means of the end of another slide. It is essential that the spreading be done with a very quick movement of the slide if the thin film is to be short. With the slides in a vertical position both thick and thin films are immersed in a bath of Giemsa about 4 cm. deep, and the end of the thin film, including the "tails," is included in the stain. The thin film receives no fixation other than that afforded by the routine drying of the thick film. Or one may make the thin film longer and allow part of it to extend above the stain. The unstained portion may be subsequently stained by Leishman's technique or any thin-film method preferred. The Giemsa stain alone, however, affords a thin film well-suited for the confirmation of any of the findings in the thick film. The staining is uniform, erythrocytes are clearly distinguishable, and Schueffner's and Maurer's dots come out sufficiently well to assist in the identification of the species of *Plasmodium*.

We make part of the thick film rather thick, averaging 50 or 75 leucocytes per field, and stain deeply, ordinarily an hour in the usual dilution of the stain; i.e., 1 drop to 1 cc. of neutral distilled water. We then decolorize in tap or distilled water until the background is purplish violet, which requires 5 to 9 minutes, depending on the acidity of the water. This method insures a clear preparation with the parasites stained in all parts of the film.

*Polychromatophilia as an index of anemia.*

The abnormal erythrocytes stainable in the manner characteristic of retained nuclear material are distinguishable as blue clouds in the thick film, and their presence may be recorded along with the routine examination for parasites. We have long used the degree of polychromatophilia or basophilia as a rough measure of anemia in a population. We distinguish four grades according to the number of
clouds per thick-film field (1/12 inch objective, 7 × ocular): I. At least one cloud but less than 20 clouds per field. II. At least 20 clouds, but less than 75. III. Seventy-five clouds or more (blue clouds almost touching one another in the thicker part of the preparation). IV. The purple or violet background of the film almost wholly lacking, as though all the color had been gathered into the very numerous blue clouds.

Grades III and IV in this region are almost always associated with parasites in the blood or with enlarged spleen. If parasites are not found in patients with these grades of anemia on one day, they often appear in a subsequent examination. Like enlargement of the spleen, polychromatophilia may be maintained by a degree of parasitism undetectable in the routine examination of blood films.

Grades I and II are often found in the blood of persons in whom there is no suspicion of malaria; for example, among infants during a non-malarious season or in a non-malarious locality. They are, however, frequently associated with malaria parasites and may be caused by malaria as well as by other diseases. They offer in this respect an analogy with the slighter enlargements of the spleen.

We find a correlation of polychromatophilia, all grades included, with the hemoglobin index as determined macroscopically by the Dare hemoglobinometer. The results for 613 children examined by both methods are shown in table 1.

Table 1.

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Grade of polychromatophilia</th>
<th>Totals</th>
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<td>30</td>
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<td>90</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
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<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Totals</td>
<td>59</td>
<td>240</td>
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The coefficient of correlation (−0.438 ± 0.022) is sufficiently high to enable one to predict from the microscopic examination of the blood
the probable index of macroscopic anemia. Such prediction would hold for groups of considerable size only, not for individuals.

In the three surveys of the sixty-five children mentioned in the beginning of this paper, we found the following percentages of polychromatophilia in order of dates of survey: with grade II, III or IV, 82, 78, 91; with grade III or IV, 39, 31, 37. The average hemoglobin determined by the Dare hemoglobinometer in the third survey was 72.4. We have usually taken the percentage of children with grade III or IV polychromatophilia as our index of "microscopic anemia," since it is perhaps the one most characteristic of malaria, at all events in this region. At the last examination the ratio of the parasite index to the microscopic anemia index was 50.8 : 37 = 1 : 0.73.

The following data on the relation of the parasite index and the index of microscopic anemia (the percentage with grade III or IV) are taken for our surveys of village children in Macedonia during the four years 1932–1935. Where the parasite index is high, the index of microscopic anemia is also high; where the parasite index is low, the anemia is low. But the ratio of these indexes (A/P) may show much variation. Generally, the higher the parasite index the greater the value of the microscopic anemia (A) in the ratio. For example, among 888 children 1 to 4 years of age, all from villages where malaria is highly endemic, the parasite index was 65.9 and the anemia index 35.8; the ratio A/P being 0.54. Among 2423 children of the same age from villages in which malaria has been much less prevalent, the parasite index was 27.7 and the anemia index 10.6; the ratio A/P being 0.38. During 1935 a sharp rise occurred in the parasite rates of two villages (Polystylo and Eratinon) of the latter group. In the spring 115 children of all ages from both villages gave a parasite index of 5.2 and an anemia index of 1.7. In the autumn of the same year, the corresponding figures for 177 children were: parasite index 56.5, anemia index 42.4. The value of the ratio A/P had changed from 0.33 in the spring to 0.75 in the autumn. In case of a fall in the parasite index of a group of villages through a period of years, the anemia index tends to fall at about the same rate.

The spleen index tends to lag both when the parasite index rises and when it falls. The microscopic anemia index, then, appears to be more sensitive to changes in the index of transmission of malaria than the spleen index, and should be a useful adjunct to malaria surveys, especially since it may be recorded along with the thick film examination, and with but little additional expenditure of time. As in all malaria indexes, race must be taken into account. For example,
Barber and Olinger (4) found a parasite index of 94.1 among 424 negro children 1 to 4 years of age in Nigeria, West Africa. The index of microscopic anemia, which in this case included grade II as well as grades III and IV, was only 3.3, hardly one-tenth of that shown by Macedonian children of the same age, even though the African children showed a much higher parasite index.

**SUMMARY.**

This paper includes: a discussion of the accuracy of the parasite index obtainable in a single malaria survey of children, and of the minimum time requisite for the examination of thick films; a recommendation that each examiner determine for himself the relation of the amount of examination (in terms of time or of microscopic fields) to the parasite percentages obtainable; a short description of a technique of preparing thick and thin films for parasite surveys; a description of the use of polychromatophilia or basophilia in malaria surveys; the method of measuring the polychromatophilia or basophilia and of determining an index of microscopic anemia; and the relation of this index to the hemoglobin index and to the parasite index.

**BIBLIOGRAPHY.**

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3. Dempwolff.  

4. Barber, M. A., and Olinger, M. T.  