uniformly produced fatal convulsions at doses of 200 mg/kg.

The results of the study on the toxicity of RC-12 administered in combination with chloroquine are also summarized in Table 3. The data presented provide no indication that toxicity is enhanced by concomitant administration of the two agents.

Vacuolation of the circulating lymphocytes was the only non-CNS reaction to the administration of RC-12, either alone or in combination with chloroquine. This vacuolation was transitory, disappearing promptly after termination of treatment. It was not associated with either hypertrophy or involution of the spleen or lymph-nodes.

Discussion

The question may now be asked what these observations on activity and toxicity indicate with respect to the potential utility of RC-12 as an antimalarial drug. It seems quite obvious from the data presented that the RC-12 *per se* has little promise as a schizontocidal or suppressive drug, and thus falls short of the goal of the much-needed substitute for chloroquine. On the other hand, it does have significant promise as a prophylactic or radical curative agent and might find at least three spheres of use in these areas: (1) when the combination of chloroquine and primaquine is not effective in causal prophylaxis, as appears to be the case where there is chloroquine-resistance; (2) when there is a need for a curative agent which can produce benefits in less than 10-14 days; and (3) when there are fears of enhanced susceptibility to the haemato-toxicity of primaquine. The promise shown by RC-12 in experimental studies pertinent to these issues seems to us to warrant evaluations at the appropriate clinical levels.

In conclusion, it should be pointed out that these studies reopen interest in an old, poorly evaluated class of compounds susceptible to substantial chemical modifications with the objectives of modifying toxicity and activity.

Statistical Considerations in the Microscopical Diagnosis of Malaria, with Special Reference to the Role of Cross-checking

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All the epidemiological and remedial activities of the advanced phases of a malaria eradication programme are based on the results of examinations of blood films for the presence of malaria parasites. It has been the experience in many malaria programmes that positive slides are not infrequently missed during microscopical examination. The tendency in most instances has been to ascribe this shortcoming either to defects in the techniques of preparation of the blood film or to the incompetence of the microscopist. The purpose of this note is to draw attention to a third and entirely different source influencing the failure to find malaria parasites in a thick blood film, namely, the " chance element ". A proportion of the positive blood films will always be missed by chance, no matter how well a film is prepared and how capable a microscopist may be.[a] These considerations have led the author to make observations on the role of cross-checking and to suggest ways of improving laboratory procedures and keeping them under constant check.

This problem of the failure to identify a positive blood sample owing to limited examination of the blood film is of particular interest to a diagnostic laboratory and is different from the one in which the blood film itself might or might not have parasites because of their low density in the peripheral blood of a patient. This note deals only with the first of these aspects, and the phrase " parasite count " is here taken to mean the number of parasites in a total of 1000 fields available for examination in a thick film of a generally accepted type.

Probability of missing a positive slide

The chance of failing to detect a positive slide depends directly on two factors, namely, the volume of blood examined in relation to the total amount on the slide available for examination and the number

[a] Dowling, M. A. C. & Shute, G. T. (1965) *Bull. Wld Hlth Org.*, **34**, 249-267.

of parasites present in this total amount. If the parasites are assumed to be randomly distributed in the blood film on a slide, it is possible to give a numerical measure to the chance that a given blood slide will be declared negative when only a sample of microscopic fields is examined. The results of such calculations [b] are presented in Table 1. The figures in the body of the table represent the probabilities that a blood slide with the parasite count stated in the first column will be missed on examining the number of microscopic fields indicated at the top.

The same table can also be used for judging situations in which one or more of the species in a mixed infection are missed. In such a case the parasite counts of each species should be considered separately and the slide treated as positive for one species at a time.

The probability of failing to detect a positive blood film decreases with increasing density of parasites when the number of fields examined remains the same. Again, for a given parasite count, this probability decreases as the number of fields examined increases while the total number of fields available for examination remains the same. These results are as would be expected on *a priori* considerations, but their practical utility lies in that they provide objective measures that are helpful in assessing the standard of microscopical examinations. Thus, if there are 18 parasites in a total of 1000 fields, Table 1 shows that, if 100 fields are examined, there is a probability of 15% that the slide will be declared negative. Stated in a different way, this means that, if a batch of 60 slides each with 18 parasites in a total of 1000 microscopic fields were examined by an expert technician examining 100 fields on each slide under the best conditions, he would, on average, classify nine of them (15%) as negative. Again, we might state that, if a team of 20 competent microscopists examine 100 fields each of a slide with 18 parasites in 1000 fields, then about three of them (15%) will declare a negative result.

Judgement of the quality of microscopic work

The recognition of the chance factor as one of the sources contributing to errors in the diagnosis of blood films leads naturally to the question: " Beyond

[b] On the assumption that each parasite has the same chance of occupying any of a total of N fields, the probability (p) that no parasites out of the total (d) will be found in a sample of n fields selected at random is given by the expression:

$$p = \left(\frac{N-n}{N}\right)^d$$

TABLE 1

PROBABILITY (%) [a] OF MISSING A POSITIVE SLIDE WHEN ONLY A SAMPLE OF MICROSCOPIC FIELDS OUT OF 1000 IS EXAMINED

Number of parasites in 1 000 fields	Number of fields examined						
	100	150	200	300	400	500	600
1	90.0	85.0	80.0	70.0	60.0	50.0	40.0
2	81.0	72.2	64.0	49.0	36.0	25.0	16.0
3	72.9	61.4	51.2	34.3	21.6	12.5	6.4
4	65.6	52.2	41.0	24.0	13.0	6.2	2.6
5	59.0	44.4	32.8	16.8	7.8	3.1	1.0
6	53.1	37.7	26.2	11.8	4.7	1.6	0.4
7	47.8	32.0	21.0	8.2	2.8	0.8	0.2
8	43.0	27.2	16.8	5.8	1.7	0.4	*
9	38.7	23.1	13.4	4.0	1.0	0.2	*
10	34.8	19.7	10.7	2.8	0.6	0.1	*
11	31.3	16.7	8.6	2.0	0.4	*	*
12	28.2	14.2	6.9	1.4	0.2	*	*
13	25.4	12.1	5.5	1.0	0.1	*	*
14	22.9	10.3	4.4	0.7	*	*	*
15	20.6	8.7	3.5	0.5	*	*	*
16	18.5	7.4	2.8	0.3	*	*	*
17	16.7	6.3	2.3	0.2	*	*	*
18	15.0	5.4	1.8	0.2	*	*	*
19	13.5	4.6	1.4	0.1	*	*	*
20	12.1	3.9	1.2	*	*	*	*
21	10.9	3.3	0.9	*	*	*	*
22	9.8	2.8	0.7	*	*	*	*
23	8.8	2.4	0.6	*	*	*	*
24	7.9	2.0	0.5	*	*	*	*
25	7.2	1.7	0.4	*	*	*	*
26	6.4	1.5	0.3	*	*	*	*
27	5.8	1.2	0.2	*	*	*	*
28	5.2	1.1	0.2	*	*	*	*
29	4.7	0.9	0.2	*	*	*	*
30	4.2	0.8	0.1	*	*	*	*
35	2.5	0.7	0.1	*	*	*	*
40	1.5	0.6	*	*	*	*	*
44	1.0	0.5	*	*	*	*	*
45	0.9	0.4	*	*	*	*	*

[a] The asterisk (*) indicates a probability of less than 0.1 %.

what level can an error in diagnosis be attributed to shortcomings in the ability of the microscopist or to defects in the techniques of staining or of making blood smears? ". Table 1 shows that a level of 44 parasites in 1000 fields should be detectable with 99% certainty. A level greater than 44 parasites in 1000 fields should be detectable with almost complete certainty. Therefore, if a particular slide with a greater parasite count has been declared negative on an examination of 100 fields, it is reasonable to conclude that factors other than chance alone have contributed to the wrong diagnosis.

This gives a means of judging the quality of microscopic work on the basis of a single blood film. A more reassuring way would be to let a microscopist examine a batch of slides of comparable parasite counts. It would then be possible to indicate limits beyond which failure should not occur if only chance factors are at play. Table 2 gives the 99% confidence intervals for the expected number of negative results, when a batch of positive slides of known count is examined.

TABLE 2
EXPECTED NUMBER OF NEGATIVE RESULTS IN A GIVEN BATCH OF POSITIVE THICK FILMS

Number of parasites in 1 000 fields	99% confidence limits		
	Number of slides examined (100 fields in each slide)		
	100	200	500
1	82-98	169-191	433-467
5	46-72	100-136	267-323
10	23-47	52-87	147-201
15	10-31	26-56	80-126
20	4-21	12-36	42-79
25	1-14	5-24	21-51
30	0-9	1-16	9-33
35	0-7	0-11	4-22
40	0-5	0-7	1-15
45	0-3	0-5	0-10

It can be seen that, when the number of parasites is, say, 30 in 1000 fields, then on examining 100 fields in each of 100 slides, even a competent microscopist may declare up to nine positive slides to be " negative ". When the number of slides declared as negative exceeds nine, one would be inclined to conclude that some factor other than chance is affecting the results of diagnosis.

The role of cross-checking, its importance and adequacy

The satisfactory working of any system of cross-checking must be judged in terms of the extent to which it achieves its purpose, which, in the present case, is to indicate and, if possible, reduce the errors in the diagnosis of blood films. Cross-checking would include observations and advice on such factors as the quality of blood films and of staining, the presence of parasites, and their species determination. However, in judging the two last factors (presence of parasites and their species) the controlling laboratory should provide an indication, whenever its diagnosis of a positive slide is at variance with that of the primary laboratory, whether this error could have been avoided by the microscopist or could have been due to chance, and, if so, in what measure.

There is an inherent advantage in any mechanism of rechecking, as this contributes an independent second examination. Thus, the results for specimens that are cross-checked are somewhat more reliable than those for specimens that are examined only once. However, in malaria eradication programmes, where only a small proportion (5%-10%) of negative slides are re-examined, this cannot constitute a great advantage. It is therefore necessary to explore ways in which cross-checking could be made more efficient. One factor would be the number of fields to be examined at each laboratory (primary and cross-checking) in order to ensure that the error in diagnosis does not exceed a predesignated level, particularly in those advanced phases of an eradication programme when one would normally expect a low count of parasites in the blood of malaria patients.

Table 3 gives the number of fields that need to be examined to ensure a 99% chance of detection at parasite counts ranging from one to 44 parasites in 1000 fields.

When a controlling laboratory confirms the diagnosis of a reportedly negative slide on examining 100 fields, there could be two possible interpretations: (1) that the slide is really negative or (2) that the slide is positive, but with less than 44 parasites in 1000 fields. In order to exclude the second possibility, a number of fields corresponding to the different parasite densities shown in Table 3 would have to be examined. However, since the density levels would not be known in the case of slides reported as negative, a decision has to be made on the basis of the expected pattern of results. Keeping

TABLE 3

NUMBER OF MICROSCOPIC FIELDS TO BE EXAMINED TO ENSURE 99 % PROBABILITY OF DETECTION OF BLOOD FILMS WITH MALARIA PARASITES

Number of parasites in 1 000 fields	Number of fields to be examined	Number of parasites in 1 000 fields	Number of fields to be examined
1	990	23	181
2	900	24	175
3	785	25	168
4	684	26	162
5	602	27	157
6	536	28	152
7	482	29	147
8	438	30	142
9	400	31	138
10	369	32	134
11	342	33	130
12	319	34	127
13	298	35	123
14	280	36	120
15	264	37	117
16	250	38	114
17	237	39	111
18	226	40	109
19	215	41	106
20	206	42	104
21	197	43	102
22	189	44	99

in view the necessity for a cross-checking mechanism to be able to detect situations where an undesirably large number of positive smears may be missed at the primary stage, it is perhaps reasonable to stipulate that a cross-checking laboratory should examine a minimum of 300-400 fields out of 1000 available fields.

Implications of a possible review of the routine instructions for the examination of blood slides

If the recommendation to increase the number of fields to be examined is adopted, this will increase the time required for the examination of a given batch of slides and therefore the number of staff engaged in microscopic work. If such an increase were incompatible with the budgetary resources of a malaria eradication service, impairment of technical performance would be inevitable. It can be seen that an increase from the normal 5 minutes (or 100 fields) per slide to, say, 7½-8 minutes (150 fields) would ensure a 99 % chance of finding a positive result with about 28 parasites per 1000 fields instead of with 44 per 1000 fields. If, on the other hand, the number of fields routinely examined is maintained at 100, even with a density of 28 in 1000 we should be prepared to miss about five cases on the average out of every 100 positive cases. Thus, while a revision of laboratory instructions on the examination of blood slides might lead to increased expenditure on staff, failure to carry out this revision is likely to result in a number of positive cases (about 5 %) being systematically missed at the laboratory. This, in turn, would lead to possibilities of focal outbreaks of malaria in the field, necessitating subsequent costly corrective operational measures. The relative importance of each of these factors in terms of expenditure (at parasite densities below 44 in 1000 fields) would seem to be a matter befitting close study. It is worth noting that the experienced staff of a cross-checking laboratory should be able to examine a greater number of fields in a given time than the staff of a primary laboratory.

Conclusions

The part played by chance factors in the errors encountered during the microscopic diagnosis of blood films needs to be recognized. It is found that, when the techniques of preparing the blood film are adequate, a parasite count of 44 in a total of 1000 thick-film fields is about the least that can be diagnosed as positive with reasonable certainty, when only 100 fields are examined. When it is reasonable to expect that, in most malaria cases, the number of parasites per 1000 fields is about 28, it is desirable to increase the time devoted to the examination of a blood slide to one-and-a-half times the standard (to examine 150 fields), and to double it and the number of fields examined when the number of parasites expected is as low as 21 in 1000 fields.

Cross-checking of blood slides should be based on a more prolonged examination of slides than at a primary laboratory. Considerations of chance can be used with advantage in assessing the quality of microscopic work.