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Correspondence

Rapid fading of carbolfuchsin stained AFB smears under extreme conditions of temperature and humidity

We were amazed to find numerous false positives when starting routine quality control of peripheral sputum smears. The controllers, our hospital laboratory technicians, could not understand such a poor performance by people to whom they themselves had given practical training. So they repeated the Ziehl-Neelsen staining on these 'false-positives' and subsequently found these smears to be clearly positive.

The phenomenon was observed in all 3 of our projects in Bangladesh, with slides coming from all our diagnostic centres. It occurs in smears from newly detected cases as well as in control smears during treatment, so with viable as well as with damaged bacilli. This finding has been confirmed by others involved in TB control in Bangladesh. We ourselves had not seen this phenomenon in countries in Africa, but fading was recently mentioned by our colleagues from Laos and from the Comores. We have not been able to find any reference to it in the literature, and none of the experts in mycobacteriology whom we asked for advice had ever heard of it.

The quality of the staining and type of basic fuchsin used was questioned. However, the stains used were rarely more than a few months old, they were made in our own centres at the usual concentrations (1% fuchsin, 5–6% phenol), and the freshly stained acid fast bacilli (AFB) always appeared strongly red. Moreover, we found that the red colour faded whatever brand of basic fuchsin was used (UCB Belgium, Fisons UK, Brixworth UK). We then ordered some New Fuchsin (Merck Neufuchsine, Germany), reportedly the best type of fuchsin and chemically different from other brands (rosanilin instead of pararosanilin). We found that fading after New Fuchsin staining was just as rapid as with any of the other brands. Another suggestion, that fading might have been caused by exposure to sunlight, was excluded since the slides were kept in closed slide boxes in a cupboard till quality control was performed.

The excellent book by Monica Cheesbrough[1] mentions, under the chapter on Mycobacteria, that there is no difference in intensity of staining between the hot and cold method when room temperature exceeds 30°C. Bangladesh is outstanding because of its extreme climate, and the original observation was made at the height of the monsoon season. We felt that the extreme combination of heat plus humidity (around 35°C and 85% relative humidity) might be the cause. Moreover, fungal growth was often seen in these smears. But although the fungi did hide some bacilli, many AFB reappeared between them after smears that had become completely negative were restained.

We therefore performed some small experiments to test our heat/humidity hypothesis. Smears containing +1 to +3 AFB (IUATLD-scale)[2] were stained with fresh carbolfuchsin solution (fuchsin at 1%), hot method, as recommended in the IUATLD manual. AFB were counted in 200–300 fields immediately. Two slides each were then placed in a tightly closed box on wet filter paper, and the boxes were put in an incubator. Relative humidity was supposedly close to 100%. Smears were subsequently checked at intervals of 1 week, and AFB counted until none could be identified any longer. As controls, 2 smears stained simultaneously were kept on silica gel and refrigerated at about 4°C, and read at the beginning and end of the experiment. Smears that had become completely negative finally were restained using the same stain, and reread.

We found it took only 2 to 3 weeks at 40°C for all AFB to become completely invisible. At 35°C, this took 3 to 4 weeks. AFB first became dark purplish, then weakly stained, until finally they became completely invisible or left only a ghost-like shape. It could take much longer for thick smears or parts thereof. After restaining, about the same number of AFB could be counted as at the start of the experiment. Over the same period of time, the control slides kept on silica at 4°C did not show any change of red colour of the bacilli, nor a clear decrease in the number of AFB detected.

Our hypothesis is that the red fuchsin stain can slowly diffuse out of the AFB, when the smear is humidified by water vapour taken up from the air. This will happen at a faster rate with higher temperatures, since the waxy mycobacterial cell wall then becomes more permeable. This is exactly the opposite of what happens during hot staining, but is slower because of the absence of the mordant, phenol. To test this hypothesis, we treated smears from which the fuchsin had already faded with acid alcohol, and then counterstained them using 0.1% potassium permanganate solution in water. Faint blue bacilli with typical morphology could subsequently be seen on the brown background. The conditions allowing the fuchsin to diffuse out of the cells and disappear in the surroundings also allow the methylene blue, present in higher concentration, to enter them.

We believe that this observation has important implications for quality control of sputum smears, now recognized as an important part of any TB control programme. Under certain climatic conditions, it may be mandatory to restain slides collected in the periph-

ery to avoid gross errors by the controllers. Restaining can be performed easily without prior attempts at destaining. Slides are simply cleaned in xylene, whereafter Ziehl-Neelsen staining is performed as usual.

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[A version in French of this letter is available from the IUATLD Secretariat in Paris.]

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Wrong drug used for tuberculosis

Thus proclaimed the banner headline on the front page of Malawi's most widely circulated newspaper, the Daily News, on 13 September, 1994.[1] The article went on to outline the life-threatening side effects of thiacetazone to its 'lay' audience and highlights the importance of the debate which was recently re-ignited by three articles in a recent issue of Tubercle and Lung Disease.[2–4]

Current addresses notwithstanding, Elliott (in Zambia) and I (in Malawi) have had extensive clinical experience in recent years in countries where human immunodeficiency virus (HIV) seroprevalence in tuberculosis (TB) patients is of the order of 70%.[5,6] I am sure that Elliott's alleged emotionalism stems from these clinical experiences. The terrible suffering of patients with toxic epidermal necrolysis secondary to thiacetazone cannot be quantified. Those that survive are left physically and emotionally scarred as are their families and their care givers. I vividly remember each of the patients affected by this particular side effect of TB treatment. I remember the hours and days of dedicated care by relatives and nursing staff and the huge strain on resources. I remember exhausting the supplies of intravenous fluids and using the last prednisolone tablet available in the hospital. In particular, I remember a patient who became a close friend and later died from a thiacetazone-induced rash. At his funeral, speeches were made accusing me of causing his death and warning the several hundred mourners not to go to the hospital for TB treatment because they would surely suffer the same fate.

So much for emotions. The rational side of the debate also points to a multitude of problems with thiacetazone. Rieder and Enarson[3] pointed to a lack of information from non-referral centres in the context of model TB control programmes in sub-Saharan Africa. St. John's Hospital, Mzuzu, Northern Malawi, is such a centre, and Malawi has a model programme. In my cohort of one hundred and eighty-seven Mzuzu TB patients,[6] skin rash was more common than previously reported from larger urban centres. Thirty-three percent of patients developed a rash with 30% of rashes severe enough to cause cessation of TB treatment. All of these severe rashes were in HIV seropositive patients. It is true that the mortality rate directly attributable to thiacetazone rash is low if appropriate treatment is available and administered promptly. However, the risk ratio of death after two years follow-up for those with severe rash, after adjustment for HIV serostatus, was 2.0 (95% confidence interval, 1.2, 3.3., $P = 0.01$).

Thus to argue for the retention of thiacetazone based solely on the cost per averted death (due to rash)[4] risks missing the true cost of the use of this drug. A wider definition of costs and benefits of various TB treatment regimens as suggested by Elliott and Foster[2] should be applied to future economic analyses. The toxic effects of thiacetazone are widely known in sub-Saharan African communities. Perceived risks often far outweigh those found by quantitative analysis. The effects on TB control via decreased detection of TB and compliance with TB treatment due to fear of this drug have yet to be addressed.

It is time to halt emotion in the debate by halting the debate. Thiacetazone should be replaced by ethambutol in all TB regimens in sub-Saharan Africa.

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