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Letter to the Editor

TO THE EDITOR:

Various chemical assay methods have been described for pyrithion salts since the introduction of zinc pyrithion (ZPT) as an antidandruff agent in 1963 (1, 2 and the references therein). However, there does not appear in the literature to be any simple, convenient method of determining ZPT *in situ* on the hair. The demonstration of ZPT binding to hair and the development of a method for assessing subsequent release during rinsing are the subjects of this letter.

250 ml Sabouraud-dextrose agar (Oxoid) inoculated to give approximately 1×10^6 cells/ml *Candida albicans* NCPF 3179 (ATCC 10231) were poured into a 25-cm square antibiotic assay plate giving a depth of approximately 4 mm. Eight ZPT aqueous suspensions (Pyrion-Chemie, W. Germany) were prepared in doubling dilutions between 200 and 1.56 $\mu g\ ml^{-1}$, and 0.1 ml of each suspension was placed in each of 8 cavities on the assay plate in a Latin square design. Following overnight incubation at 35° the mean zone diameters were calculated for the eight suspensions and the square of these values plotted (on the ordinate) against logarithm of the concentration: a linear relationship between 1.56 and 25 $\mu g\ ml^{-1}$ resulted. Slight day-to-day variation in the calibration plot occurred, but a typical line was that represented by the equation $y = 283x + 172$ (correlation coefficient 0.998, which exceeds the tabulated value of 0.991 for $p = 0.001$). Using a similar procedure, inhibition of *Pityrosporum ovale* was observed, but the zones, although larger, were less clearly defined and growth was slower.

100 mg finely cut unwashed human hair was immersed in 5 ml supernatant liquid from a 20 mg ml^{-1} ZPT aqueous suspension for 15 min at room temperature and centrifuged at 2000 $\times g$ for 10 min. The hair was washed once in 5.0 ml water, recentrifuged, suspended in 1.0 ml water, and 0.1 ml of this suspension placed in 8 wells on the assay plate. The mean inhibition zone created was equivalent to that resulting from a 5.5 $\mu g\ ml^{-1}$ suspension. No antimicrobial activity was observed either in suspensions of untreated hair or in the water used for rinsing hair treated and centrifuged as described above. This indicates that the activity in treated suspensions was due to ZPT adsorbed onto the hair rather than to "carry-over" in the suspending medium. When 8 such 100-mg hair samples were individually determined, the coefficient of variation for the calculated equivalent ZPT suspensions was 11.9% (mean 2.0 $\mu g\ ml^{-1}$).

Four 100-mg hair samples were similarly treated but subjected to one, two, three, and four $\times 3$-ml water washes respectively. This resulted in the second, third, and fourth samples exhibiting zones equivalent to ZPT concentrations which were 88, 55, and 50% of that due to the single-washed sample. The results indicate that ZPT is adsorbed onto hair and is subject to limited removal on repeated washing. The observed zones of inhibition must, therefore, have been created following the desorption of ZPT from

the hair during the incubation period. The ZPT levels in the wash water were below the limits of detection as a consequence of the relatively large wash volumes used. The observation that approximately 50% of the ZPT is still associated with the hair after three washes is in agreement with that of Okumura *et al.* (3) who concluded that the ZPT was not easily removed from hair by a following application of a plain shampoo or plain cream rinse.

The publication by Okumura *et al.* (3) is one of the few in which ZPT determinations on hair or skin have been described. Their method, although sensitive and reliable, was based upon the use of ZPT radiolabelled on the sulphur atom, a material which is not readily available in most laboratories. Results of the direct measurement of antimicrobial activity of ZPT on calfskin have been published (4), but the experimental details were not included and the untreated calfskin itself clearly had inhibitory activity towards certain organisms.

The method described in this letter is simple, reproducible, and uses readily available materials. It is suggested that the method is likely to be of value in, for example, the rapid assessment of ZPT leachability from hair following application of different shampoo formulations and the factors influencing this process.

REFERENCES

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