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Do nit removal formulations and other treatments loosen head louse eggs and nits from hair?

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Abstract. Eggs of the head louse, *Pediculus capitis* De Geer (Phthiraptera: Pediculidae), are difficult to remove because the female louse fixes them to hairs using a proteinaceous secretion that hardens within seconds. The persistent eggshells are harmless but unsightly and are often mistaken for an active infestation. Combing with a fine comb (nit comb) does not readily remove the eggs or empty eggshells because of the resilience of the fixative and both folk remedies and medical products have claimed to facilitate their removal. Measurement of the force required to initiate sliding of the egg fixative using a slip-peel tester was unable to detect evidence that any of three products which claimed to have egg-loosening properties (Step 2™ Nit Removal System, Clear® Lice Egg Remover, RID® Lice Egg Loosener Gel) had any activity or exerted any effect on the egg fixative beyond the lubricating effects conveyed by water or conventional hair conditioner.

Key words. Conditioner, enzyme, head louse, louse egg, nit, nit remover, slip-peel tester.

Introduction

Treatment of infestation by the head louse, *Pediculus capitis* De Geer (Phthiraptera: Pediculidae), is relatively easy using topically applied preparations that kill the insects and their eggs. However, head lice fix their eggs to hair shafts using a cement-like substance secreted from accessory glands to the oviduct (Buxton, 1947). This material hardens within seconds to form a resilient holdfast that retains the empty eggshell in position for months after the young louse has emerged. As hair grows, the empty eggshells, known as nits, grow out with it.

Although nits are harmless, the empty shells are refractile and appear white against the colour of the hair. Consequently, as they move further from the scalp, nits become more easily detected and cosmetically unacceptable because, apart from being unsightly, they also reveal that the person has had a louse infestation, which may result in stigmatization. Currently, screening for head lice infestation in schools largely relies on finding nits, simply because lice can be difficult to see. As a result it is not uncommon for children with extinct infestations, but with visible dead eggs and nits, to be treated unnecessarily in the mistaken belief that the eggshells represent a continuing problem (Williams *et al*., 2001). Therefore, in some territories nit removal is not only a cosmetic issue, but may be considered a therapeutic requirement, such as after the use of products that have limited activity against louse eggs. In addition, some school boards still maintain a 'no nit' policy, which precludes children with any nits or louse eggs from attending school until all have been removed (Williams *et al*., 2001; Mumcuoglu *et al*., 2006).

Nit removal has always constituted a challenge that frequently defeats the best efforts of most parents and guardians (Greene, 1898). Most efforts to facilitate removal have been based on designing combs with closely spaced teeth that are intended to drag the nits along the hair shafts as the comb is pulled through the hair. However, many so-called nit combs are not adequate for the purpose. Even when the comb is properly designed, using it requires considerable time and effort if all nits and eggs are to be removed, even from relatively short hair (de Souza Bueno *et al*., 2001; Speare *et al*., 2007). Therefore, most consumers would welcome a treatment that facilitates nit removal.

In our laboratory we have conducted several evaluations, since our first test in 1989, of putative nit-loosening or nit-removing products (Burgess, 1995). This report summarizes our findings.

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Materials and methods

Washed, unbleached, untreated human hair of European origin obtained from a wig maker was used for egg removal experiments. Hair samples for measurement of diameter were taken from the posterior parietal region of the scalps of colleagues and associates. Small locks of hair, minimum 50 hairs per sample, were pulled between a thumb and forefinger coated with adhesive to produce a flat band. Transverse sections 1 mm thick of bands of glued hairs were mounted on a microscope slide so that the cut face of the hairs was facing the microscope objective. Prior to taking measurements, changes in the depth of field were used to check that each block of hairs was perpendicular so that the cut face of each hair was square to the line of sight. The shaft diameters of 40 hairs from each sample were measured using a Wild M3Z stereomicroscope fitted with an eyepiece graticule to determine the longest and shortest diameter of each hair.

The natural orientation of louse eggs on hairs was determined by supplying female head lice, *Pediculus capitis* De Geer, maintained in arm boxes on volunteers, with small locks of hair from different sources. Hairs were removed from the boxes every 8 h to be counted and the orientation of eggs on the hair shaft was determined by observation using the stereomicroscope.

Louse eggs and nits for testing the force required to remove the eggshell from the hair were obtained by supplying laboratory-reared human body lice, *Pediculus humanus* L. (Phthiraptera: Pediculidae), with 60-mm long strands of human hair spread over the bottom of a glass crystallizing dish. Human body lice were used because the egg cement material produced by body lice is essentially the same as that of head lice (Burkhart & Burkhart, 2005), was more readily available, and could be produced under controlled conditions with identical ageing characteristics for each sample, whereas head louse eggs and nits collected in the field are of different ages and could have been subjected to considerably different chemical and physical conditions in the period prior to collection.

Recently fed lice were permitted access to the hair over a 24-h period. Eggs were used within 48 h, but if nits were tested the eggs were incubated at $30 \pm 2^{\circ}$C and $50 \pm 15\%$ relative humidity until all nymphs had emerged. For one baseline test on 40 untreated louse eggs, the length of the cylinder of egg cement material was measured, using the stereomicroscope, prior to measuring the removal force.

In 1989 we identified a test apparatus with adequate sensitivity to measure the force required to cause louse eggs and nits to slide along hair shafts (Burgess, 1995). This device, the slip-peel tester, is normally used to measure either friction of surfaces against a standard or the force required to remove adhesive tapes from standard surfaces. Initially we used a model SP-101, but more recently we have used a model SP-2000 (IMASS, Inc., Accord, MA, U.S.A.).

We devised a system for gripping the hair and holding the louse egg so that it could be slid along the hair shaft without applying other forces. We found that the base of a louse egg, still attached to a hair, would fit into the aperture of a 1-μL disposable Drummond Microcap™ tube (Drummond Scientific Co., Broomall, PA, U.S.A.). Tubes were fixed across glass microscope slides, using epoxy resin adhesive, and were taped to the platen of the slip-peel tester at an appropriate level relative to the force measurement sensor.

For each test, a single egg or eggshell on a hair was selected and the shaft of the hair threaded through the Microcap™ tube, with the free end of the egg facing away from the tube (Fig. 1). The end of the hair that passed through the tube was then clamped to the sensor using a modified bulldog clip so that, as the hair was drawn through the tube, the base of the eggshell was pulled against the aperture of the Microcap™ tube at the opposite end to the sensor.

The slip-peel tester operates a rolling platen that moves away from the sensor (Fig. 2), bringing the egg into contact with the Microcap™ tube and applying force to the base of the eggshell. When the force applied exceeds the inertial grip of the cylinder of cement substance on the hair shaft, the egg starts to slide.

The force required to move an eggshell along the hair has two components. The first, which initiates sliding and is known as 'static peak force', is a record of the maximum inertia involved in movement. The second component, the force required to continue the sliding process, theoretically has a lower inertial component and is recorded as 'average force', which is a 'smoothed out' reading of the continuous pressure requirement over the period of continued slippage.

Various preparations have been marketed with claims that they facilitate the removal of louse eggs and nits by 'breaking the bond' between the tube of fixative material and the hair, thus easing the initiation of sliding. Consequently, for evaluation of this property we measured the static peak force only. In order to obtain baseline characteristics, we measured static peak force for a variety of hair states, including: dry; wetted with water; coated with olive oil; treated with a simple cetyl alcohol-based hair conditioner (Tesco Value Conditioner; Tesco PLC, Cheshunt, U.K.) (Fig. 3), and after a pediculicide treatment using a popular over-the-counter synergized pyrethrum shampoo (RID® Lice Killing Shampoo; Pfizer Inc., Parsippany, NY, U.S.A.). In most cases the products under test were designed to be used in the North American market. Products were applied according to the package instructions for use, which included a pre-wash using a commercial louse-killing shampoo in some cases. When a product was required to be washed off, this was achieved by rinsing in three changes of tap water after which excess fluid was blotted off.

A minimum of 40 eggs/nits on hair per treatment method were evaluated at each test session. The measurements of force required to initiate sliding of the eggshells or to maintain sliding for each treatment were compared with those of the appropriate control batch or with other test batches using the Mann–Whitney test. Other comparisons were made using chi-squared analysis.

Results

We found that hairs of Indo-European origin (Caucasoid ethnicity, six samples) were predominantly (62–83%) oval in cross-section, with a smaller proportion of round or broader

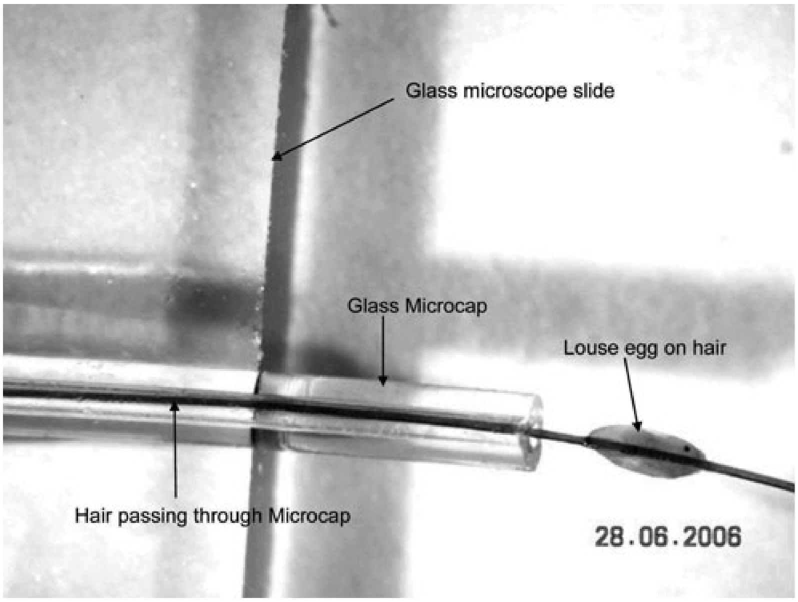

Fig. 1. A louse egg on a hair mounted in position for testing with the hair threaded through the Microcap™ tube.

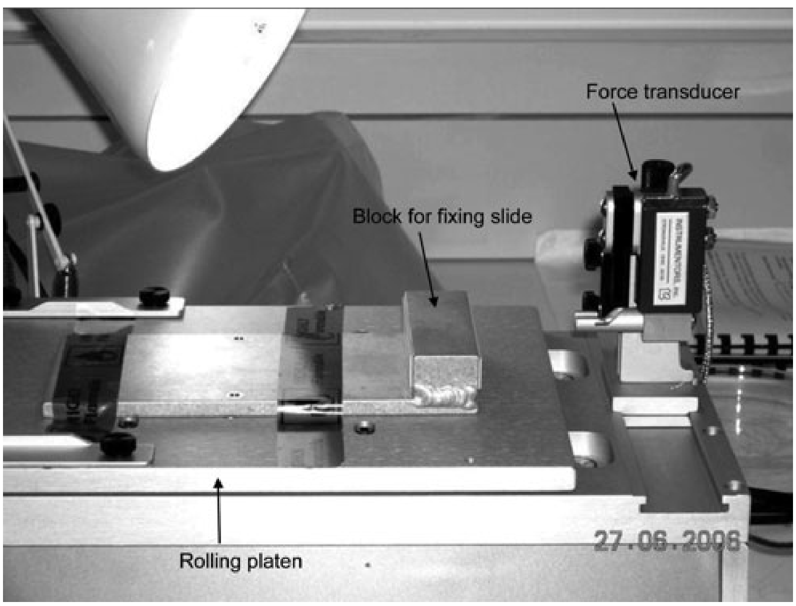

Fig. 2. The slip-peel tester platen with a block for attaching microscope slides with attached Microcap™ tubes.

oval hairs. The actual proportions of oval and rounded hairs varied with the individuals from whom they were taken and there was also some variation within an individual depending upon the part of the scalp from which the hairs were taken. Hairs taken from eastern Asian individuals (Mongoloid ethnicity, two samples) were predominantly (>90%) round in cross-section. Mean hair diameter (longer value first) of the sample examined was (106.5 ± 15.9 μm by 83.8 ± 13.3 μm) for European hair (range 51–126 μm). The sample of Asian hair was finer and more consistent in diameter (mean 57.0 ± 6.3 μm by 51.4 ± 4.9 μm).

We measured static peak force and average force for sliding of louse eggshells along both oval (European) and round (Asian) hairs after the hair had been wetted with water for 10 min and excess moisture blotted off. There was a significant difference ($P < 0.001$) between the two hair types in measured peak force, with a high proportion (30.0%) of the eggs beginning to slide along the round hair without exerting sufficient force to register on the sensor (mean peak force, oval hair, 48.27 mN, standard error of the mean [SEM] 6.49; mean peak force, round hair, 9.86 mN, SEM 1.85) (Fig. 4). Similarly, there was a significant difference ($P < 0.001$) between the two hair types in the average force required to maintain the sliding of louse eggshells a distance of 40 mm along the hair shaft. We found that the average force for round hairs was mostly no different from the peak force (mean average force 6.48 mN, SEM 0.99; $P = 0.357$), but the average force for oval hairs mostly exceeded the peak force, although the difference was not statistically significant (mean average force 60.75 mN, SEM 7.99; $P = 0.264$).

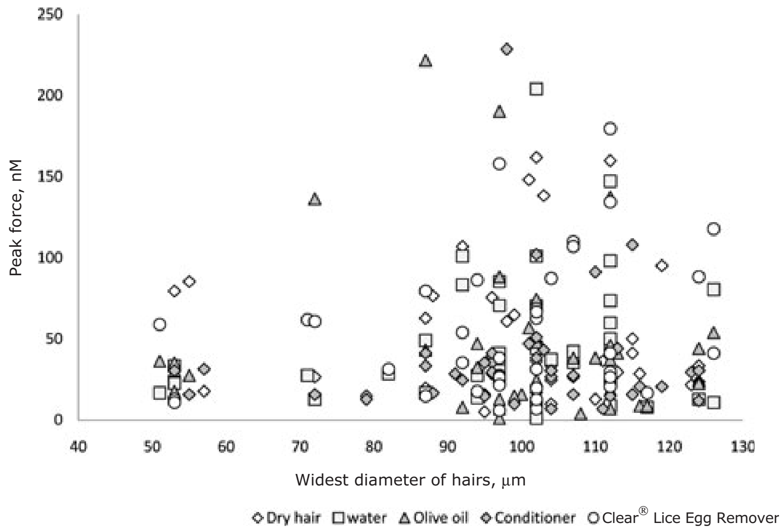

Fig. 3. Comparison of static peak forces required to move louse eggs/nits following various treatments, in comparison with untreated (dry) hair.

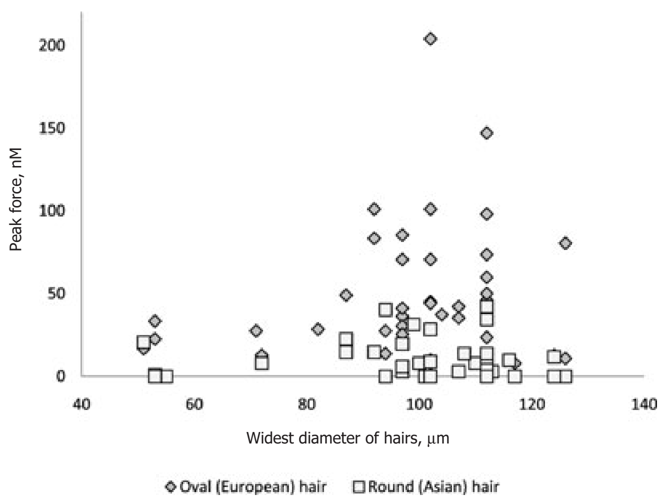

Fig. 4. Comparison of static peak forces required to move louse eggs/nits on oval and round hair shafts.

There was no detectable relationship between the peak force or the average force with either the maximum diameter of the hair shaft or the length of the cylinder of louse cement material used to hold the eggshell in place on the hair. However, of 1579 head louse eggs laid on oval hairs, the significant ($P < 0.001$) majority, 1358 (86%), were laid against the broader face of the hair shaft and the remaining 14% were applied to the narrower side of the ellipse or in some intermediate position.

We found that orientation of the egg on the hair had potential to influence the average force measurement during the sliding process. As hairs were subjected to increased tension, the friction resistance of the cylinder of louse fixative material against the hair shaft tended to cause the hair to twist. As the louse eggshell was held firmly by the end of the glass Microcap™ tube, any rotation of an oval-shaped hair within an oval-shaped aperture inside the cylinder of louse fixative (see Fig. 5) increased the force required to maintain the slippage of the eggshell along the hair.

We found there was no statistical difference between the baseline treatments, although there was a non-significant trend for easier initiation of sliding after using conditioner compared with on dry hair ($P = 0.081$) and after using conditioner compared with RID® Lice Killing Shampoo ($P = 0.078$) (i.e. the pediculicide alone made moving the eggshells slightly more difficult). There was also no detectable difference in the peak force required to move hatched compared with unhatched eggshells.

Measurements of peak force were made for three products that each claimed to facilitate loosening of louse eggs and nits and compared with measurements taken for a substance traditionally used to loosen them, namely, vinegar. The first product was a crème rinse containing 8% formic (methanoic) acid, Step 2™ Nit Removal System (Genderm Corp., Northbrook, IL, U.S.A.). We found there was no difference in initiation of sliding of louse eggs and nits on untreated dry hair compared with those on hair treated with the methanoic acid preparation (Table 1). Similarly, Clear® Lice Egg Remover (Care Technologies, Inc., Darien, CT, U.S.A.) also required a non-significantly higher static peak force than that required to move eggs and nits on dry hair. Malt vinegar (a commercial preparation of approximately 5% ethanoic acid) was found to make the initiation of sliding marginally easier than the

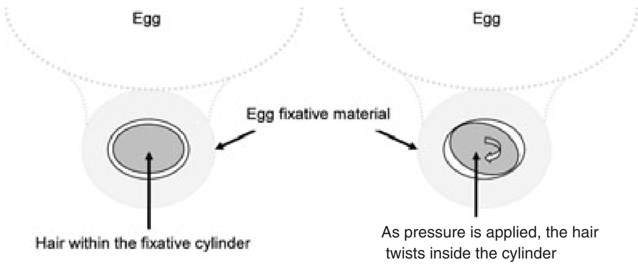

Fig. 5. Diagrammatic representation of an oval hair twisting inside the cylinder of louse fixative material as pressure is applied to a louse egg/nit during sliding.

Table 1. Comparison of static peak force required to initiate sliding on hair under different treatments.

			P-value vs.:		
Hair treatment	Mean static peak force	SEM	Dry	RID® shampoo	Conditioner
Dry	50.87	6.65	NA	0.597	0.081
Water	48.27	6.49	0.844	–	–
Olive oil	44.25	7.62	0.235	–	–
Conditioner	36.85	6.09	0.081	0.078	NA
RID® Shampoo	37.71	3.14	0.597	NA	0.078
Step 2™*	59.52	6.70	0.958	–	–
Clear® Remover	52.81	6.78	0.939	0.386	0.077
RID® Loosener	25.03	3.14	–	0.00579	0.143
Hand cleanser	11.25	2.78	–	2.14E-08	–

*This test was conducted using a different dry control batch with a mean static peak force of 59.13 (SEM = 6.83).
SEM, standard error of the mean.

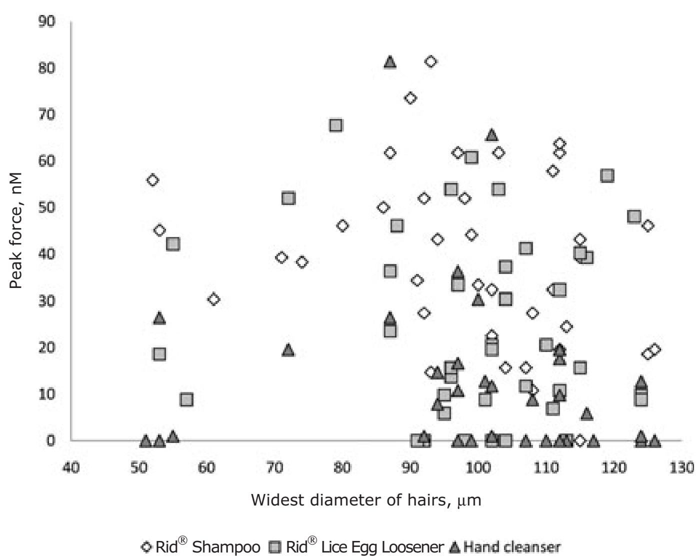

Fig. 6. Comparison of static peak forces required to move louse eggs/nits following treatment with RID® pediculicide Shampoo, RID® Lice Egg Loosener, and an unnamed hand cleanser.

two products, but was not distinguishable from using water alone.

One commercially available preparation, RID® Lice Egg Loosener Gel (Pfizer, Inc.), was found to exhibit a significant effect ($P < 0.006$) to reduce the peak force required to dislodge nits when applied after treatment with RID® Shampoo compared with RID® Shampoo alone (Fig. 6).

However, all products marketed as nit removal treatments, and all experimental formulations supplied by various sponsors at other times, were eclipsed in their effect by an unidentified amphoteric surfactant (Miranol®)-based hand-cleansing gel, which gave static peak force values that were highly significantly lower ($P < 0.0001$) than those required after using RID® Shampoo (Fig. 6). Treatment with this preparation also resulted in a significantly ($P < 0.0001$) lower average force as the eggs and nits slid along the hair shaft, indicating a high degree of smoothing of the hair shaft surface and lubrication to prevent the hair from twisting inside the cylinder of louse egg cement. This material was screened as an anonymous sample and further development of the product was terminated after a company takeover. We concluded that unless a preparation applied to the hair exhibited exceptionally good lubrication of the hair surface, it did not significantly facilitate sliding of the louse eggshell along the hair shaft.

Discussion

Lice bind their eggs and nits to hair shafts by means of hardened accessory gland secretion, shown to be primarily proteinaceous in nature by Carter (1990) and confirmed by Burkhart *et al*. (1998, 1999a, 1999b). The material appears to consist of aligned polymerized peptides (Schmidt, 1939) and it has been suggested that the proteins form β pleated sheets (Burkhart & Burkhart, 2005). The fixative holds the egg firmly in position on the hair during its incubation period, but has such resilience that it persists for a long period after hatching and is not dislodged by hair washing or normal grooming. However, if the egg or nit is forcibly slid along the hair, there is no damage to the hair structure or the cylinder of louse fixative material and no fragments of scales from the hair shaft have been detected adhering to the inside of the cylinder of 'glue', although small ridges have been seen inside the cylinder where the material was moulded against the scales of the hair surface (Burkhart *et al*., 1999a; Burkhart & Burkhart, 2005). Consequently, there is no evidence of chemical adhesion and it appears only that the fixative acts as a clamp created as the secretion hardens by polymerization.

Various manufacturers have developed formulations that are described as acting to cause the cement material to release its grip. Some of these have been formulated based on a false understanding of the chemical nature of the cement-like material, which was described as being chitinous in nature by Barat & Scaria (1962). The 8% methanoic acid preparation was developed based on this premise, although there is no evidence that methanoic acid is chitinolytic, as suggested by the manufacturers (Defelice *et al*., 1989), especially as it is a major component of several insect venoms. Moreover, it is difficult to imagine how such a low concentration of the acid could be effective in disrupting the highly resilient fixative when applied for only 10 min. In the manufacturer's clinical study, combing out nits using the 'nit removal system' was found to be highly significantly more effective than combing following treatment with shampoo (Defelice *et al*., 1989). However, the 'nit removal system' included a metal nit comb, whereas the comparator combing was performed with a plastic comb with relatively widely spaced teeth. Hence this result was not surprising because, as Speare *et al*. (2007) have demonstrated, metal combs are superior to plastic combs in nit removal and thus there is no evidence that the methanoic acid rinse contributed to the process in the study by Defelice *et al*. (1989). The product was withdrawn by the manufacturer after a short period on the market.

Similarly, it is difficult to understand the claims made by another manufacturer (Care Technologies, Inc.) (Upton, 1999): 'Clear® Lice Egg Remover is a vegetable derived enzyme system that makes nits easier to remove after treatment by loosening the glue that bonds nits to hair… A targeted enzyme solution, it rapidly attacks and loosens lice egg cement.' However, the list of enzymes given on the packaging, 'oxidoreductase, transferase, lyase, hydrolase, isomerase, ligase', is non-specific and includes enzyme groups unlikely to act on a polymerized protein and unlikely to have activity within the 10-min application time.

Despite implied claims, none of the manufacturers were able to adequately demonstrate efficacy either *in vivo* or *in vitro*, which led the US Federal Trade Commission to issue an Order to one manufacturer that it '..shall not make any representation, in any manner, expressly or by implication, that such product loosens, unglues, or otherwise detaches lice eggs from the hair, unless the representation is true and, at the time it is made, respondent possesses and relies upon competent and reliable scientific evidence that substantiates the representation' (Federal Trade Commission, 1998).

Our results showed that two preparations, Step 2™ and Clear® Lice Egg Remover, had no demonstrable effect on the louse egg fixative when used according to instructions. Consequently, nit removal using these products would be no easier than using a comb alone on dry hair. Simple conditioner was found to have a limited effect in reducing static peak force, but its main benefit in use was that it reduced the average force during the sliding process by acting as a lubricant between the cylinder and the hair shaft. Soaking the eggs/nits in vinegar, which is widely believed to facilitate nit removal, was no more effective than soaking them in water.

RID® Lice Egg Loosener gel (now marketed as RID® Egg & Nit Comb-Out Gel; Bayer HealthCare LLC, Morristown, NJ, U.S.A.) also claimed enzyme action. The active components included cabbage extract and pineapple extract, of which the latter has a recognized cysteine proteinase activity that has been demonstrated to soften or break down silk gum sericin, a protein that binds the fibroin silk strands in the cocoons of oak tasar silk moths (*Antheraea proylei*, J.) (Singh *et al*., 2003). The product also contained a 22-carbon cationic conditioning and detangling agent, behenamidopropyl dimethylamine behenate, which reputedly has better wetting and conditioning properties than cetyl alcohol. However, any enzyme activity brought about by this product is likely to be extremely limited during the short application time and the lubricant activity of the conditioning agent is unlikely to be significantly different from the conditioning effect of other widely used materials such as the ceto-stearyl alcohols. Although it showed a significant advantage over combing dry hair, or hair pre-treated using pediculicide shampoo alone, it did not show a significant advantage over simple conditioner ($P = 0.143$).

We have been able to demonstrate experimentally that preparations that claim activity to loosen head louse eggs and nits from hairs and facilitate their removal did not fulfil their claims and were no more effective than a simple conditioner. It was not surprising that we could detect no significant advantage in using the products as the louse fixative material is not a chemical glue, but merely clamps the eggshell to the hair, so the basis upon which the products were developed was scientifically flawed.

Conclusions

We conclude that so-called 'nit removing' products have no effect in terms of loosening the grip of the cement-like fixative secreted by the female louse to hold her eggs in place on the hair. None of the products tested demonstrated any detectable chemical or physical activity beyond lubricating effects indistinguishable from those found for widely used toiletry or cosmetic products. This is consistent with the finding that the fixative material is a simple physical holdfast rather than a chemical adhesive. Therefore, we conclude that the most effective means for loosening louse eggs and nits, and for maintaining the sliding of the eggshells along the hair shaft, is likely to be achieved by simple lubricating materials with relatively low surface tension that can smooth the surface of the hair and perhaps infiltrate any spaces between the cylinder of the egg and the hair shaft. There appears to be no justification in purchasing products specifically marketed for this purpose.

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