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HUMAN LICE AND THEIR CONTROL

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■ **Abstract** Current research on human louse biology has focused on the long-standing debate about speciation of head and body lice but using new tools of DNA and enzyme analysis. These studies have indicated that head and body lice from the same geographical zone may be more closely allied than insects inhabiting the same ecological niche in other regions. However, the majority of research over the past decade has involved clinical aspects including transmission, treatment, and the appearance and identification of resistant strains within populations of lice. Despite advances, there is a need for a better understanding of louse biology, as existing therapies fail and lice remain potential vectors of disease for millons of people.

INTRODUCTION

Following the early successes of using DDT for the control of body lice, *Pediculus humanus*, during the typhus outbreak in Naples in 1943, it was anticipated by many medical entomologists that the days of these parasites, and the diseases they carried as vectors, were numbered. The sense of euphoria was such that some were prompted to make rash statements such as, "The efficiency of DDT in controlling body and head lice. . .is so great that perhaps we have now seen the last great typhus epidemic" (26). Despite the rapid appearance of resistance to DDT in body lice and subsequent development of resistance to other insecticides, most people remained optimistic that lice were conquerable as pests, or at least manageable, and were no longer considered to be of real public health importance. As a result, few researchers showed any interest in studying these insects from the 1950s through the 1980s. Lice were not fashionable and it became virtually impossible to obtain funding to work on them other than from pharmaceutical or chemical companies as part of their support for products used to control lice.

However, during the 1990s the prevalence of lice increased worldwide as a result of resistance and other factors, and the study of human pediculosis took on a new lease of life with numerous new investigators in the field. The increased interest resulted in the organization of the Second International Congress on Phthiraptera in Brisbane, Australia, 8–12 July 2002 (45), nearly 30 years after the first Congress

(76)! This review summarizes the recent significant changes to our knowledge and understanding of these fascinating parasites.

BIOLOGY

Taxonomic Discussions

Work on the fundamental biology of human lice has not figured prominently since before World War II. This is not because our knowledge of the insects is comprehensive, but rather many of the problems associated with louse control today result from lack of information about some of the most basic factors of physiology, survival on and off the host, longevity, fertility, fecundity, and behavior. This lack of knowledge extends even to deciding whether the head louse, *Pediculus capitis*, is a distinct species or subspecies of the clothing or body louse, *P. humanus*. Until recently, such distinctions were made solely on grounds of morphology and behavior. Busvine (24) examined lice taken from the heads and clothing of people with double infestations. By measuring sclerotized parts of the cuticle, such as the length of claws and limb joints not affected by post-mortem shrinkage, Busvine identified two distinct populations of lice, with only a small overlap. Although head lice and body lice are able to interbreed in vitro, he concluded that the populations studied represented two distinct species on the grounds that in the wild their habitats are distinct and they would be unlikely to meet (24). This position was also adopted in a 1995 editorial commentary in *Medical and Veterinary Entomology* in which it was concluded that "Eco-epidemiologically, infestations of head or body lice do not arise from each other. . .proving that they are an allopatric pair of apomictic species" (100). However, I have been informed of several observations (C.M. Brown, personal communication) of head lice from heavily infested scalps taking residence in clothing on the upper body of children who habitually wore the same underclothing for several days at a time. These lice appeared settled in the clothing and made no attempt to return to the head, as displaced head lice would normally do. Consequently, Busvine's proposal (25) that the head louse is the ancestral form that colonized clothing and subsequently became ecologically isolated may be correct.

Taxonomic Investigations

Recent investigations (3, 52, 53) at the subcellular level have begun to provide information to elucidate this long-term question. Examination of mitochondrial DNA from both head and body lice from nine countries found 10 haplotypes that differed by between one and five base pairs at 11 nucleotide positions. Of these, three haplotypes were shared between head and body lice and, although tests of frequency of these haplotypes found significant differences between the two forms of lice, greater differences were found between lice from different countries. The constructed phylogeny suggested that head and clothing lice are conspecific and not

from reciprocally monophyletic lineages. One common and widespread haplotype was identified as possibly being ancestral. Others isolated only from provinces of China were not considered ancestral but, as they were found in both head and body lice, were thought to provide evidence of conspecificity (53).

Amevigbe et al. (3) compared isoenzymes from head and body lice from France and former French colonies in Africa with those from laboratory body lice originating from the United States. Only 2 of 28 enzymes examined showed electrophoretic variation. Phosphoglucomutase had three alleles that occurred in laboratory lice and some European head lice. Only two alleles occurred in African head lice and those from Madagascar exhibited but one. An esterase that behaved like cholinesterase had four alleles. One was isolated only from head lice from Mali, and a second was found only in specimens from Senegal. The other two alleles showed a balanced distribution in head lice from France and Madagascar and body lice from France and the laboratory colony. These results appear to provide evidence of conspecificity and a similar variation of alleles in the geographically isolated populations similar to the mitochondrial DNA evidence found by Leo et al. (53).

Phylogeny of louse populations is no doubt complex and influenced by epidemiology of infestation. As people have become more mobile over recent decades, both within and between countries, the likelihood of mixing louse populations has also increased. The two internal transcribed spacers (ITS) of ribosomal DNA have been used as markers of other arthropod populations to track such movements but not always successfully (108). However, PCR amplified sequences from lice revealed that all 67 lice examined from four countries had several ITS2 types, and construction of a limited phylogenetic tree showed that none of the sequences from single insects were monophyletic. Consequently, ITS2 is unlikely to be useful as a marker for human lice (52).

Biological Factors Influencing the Epidemiology of Infestation

There is still a need for elucidation of fundamental elements of louse biology. Much of the literature disseminated to professionals and the public contains imprecise and sometimes misleading information, often apparently copied from one publication to another without critique. Two such elements are longevity, both on and off the host, and fecundity of lice, factors that can have a profound influence on the epidemiology of the infestations.

SURVIVAL OFF THE HOST Many people, particularly in North America, spend considerable time and money not only treating louse infestations on heads but also treating and cleaning their home environment. Despite this widespread practice there is little evidence, if any, in its support. Meinking & Taplin (60) have reported observing lice on various objects while working in Panama, usually in communities with high levels of infestation that facilitate displacement of lice from their normal positioning. Similarly, I saw lice crawling off the heads of individuals

with heavy infestations in a tropical environment where differentiation between the temperature and humidity of the head and an inanimate object is not obvious (13). Because mainland areas of the United States reach similar or greater temperatures during summer, it has been claimed that those areas are likely to have conditions that facilitate transmission of head lice onto fomites (inanimate objects) (60). However, use of air conditioning would lower not only the temperature but also the humidity, a combination that would discourage lice from leaving their hosts and diminish their survival. In Queensland, Australia, an area just as warm and humid as mainland United States, a study specifically looking for lice on the floors of classrooms in elementary schools found no sign of the insects in just the circumstances where advocates of fomite transmission would suggest there is the highest risk (92). In contrast, experiments presenting hairs to lice demonstrated that insects are reluctant to transfer from one hair to another unless they are moving slowly in the right spatial alignment (27). The authors concluded that lice are most likely to rely on head-to-head transfers and that fomite transmission is less likely, although at least one author passing comment on the study chose to interpret the results in exactly the opposite way without any clear explanation of why he came to such a conclusion (21).

Head lice deliberately removed from their hosts ceased movement in less than 55 h (mean 21.3 ± 12.1) (29), or 35 ± 1.7 h at 18°C or 24 ± 1.8 h at 26°C (47). Many lice are nonviable and are unable to feed as a result of dehydration long before they stop moving or even walking (13). This means that a louse accidentally transferred to a pillow in the morning would unlikely be viable when the host goes to bed again that night. Furthermore, Chunge et al. (29) found no lice or viable eggs on brushes and combs, and eggs deliberately removed failed to hatch at room temperatures (fluctuating between 20° and 30°C). Experimentally, at a "high room temperature" of 26°–27°C, viability of louse eggs is reduced so that less than 50% of either head or body louse eggs hatched in 9–17 days at 50% relative humidity (47, 51). Consequently, the risk of transmission of infestation by displaced lice or louse eggs is epidemiologically insignificant compared with the risks of lice transferring from one person to another during physical contact, a conclusion drawn from studies conducted in the United States more than 20 years ago (45a).

FECUNDITY The number of eggs lice lay and their longevity are important in relation to development of an infestation and its possible transmission. Lang (47) found, over 27 generations of culture, that female lice lived a mean of 31.9 ± 1.5 days, during which they laid an average of 6.6 ± 3.9 eggs daily. However, peak oviposition occurred only in younger lice, from a few days after becoming mature, and declined as the insects aged. If virgin females were inseminated, they laid eggs from $\sim 14.4 \pm 3.7$ h after mating. After insemination they produced 56 ± 6.6 viable eggs at a rate of 7.5 ± 1.4 daily, over a period of 7 to 8 days (maximum 9.5). Lang (47) noted that the fecundity of lice in his study was considerably greater than that reported from various investigations performed in the early twentieth century.

SCREENING FOR INFESTATION

Diagnosis

Diagnosis of a louse infestation is simple. Either lice are present or they are not and sophisticated methods are not required to determine this. Often the problem is one of technique, visualization, interpretation of what is found, and understanding.

ACCURACY OF DIAGNOSIS Accurate diagnosis is the key to successful identification of infestation, whether clinically or epidemiologically. It is also necessary to determine whether treatment is necessary or has been successful. Traditionally, direct observation of the scalp after parting the hair has been used to make diagnoses, and various aids to vision have been employed in attempts to facilitate the process, but none has proven successful. This problem is exacerbated by the inability of many people to identify accurately what they find in the hair. Analysis of 614 samples sent to researchers at the Harvard School of Public Health found that only 364 contained any material of louse origin, of which only 53% included a louse or an apparently viable egg. Clinical staff were also often less effective at making a sound diagnosis than parents, caregivers, and teachers, with the result that many children were treated unnecessarily (81). Much of the misdiagnosis revolved around whether louse eggs were intact, apparently with a viable embryo, or hatched (what is more correctly called a nit). Presence of nits and eggs after treatment has been a contentious issue in the United States and some other countries on the grounds that eggs may not have been killed by pediculicides and, unless all are removed, a cure cannot be confirmed (1a). This has resulted in some bizarre interpretations of the survival capability of lice in some information distributed by school boards to their families.

COMBING VERSUS SCALP INSPECTION Diagnosis by inspection has recently come under scrutiny and alternatives based on combing have proven more effective. Using a comb on dry hair, or hair dampened with only a light water mist, has proved four times more effective (25% versus 6%) in finding lice on 79 infested children and twice as fast (57 sec versus 116 sec) for finding lice during screening examinations than visual inspection (66). Others have preferred combing but using conditioning crème rinse as a lubricant, the so-called wet-combing method. When this was compared with scalp inspection, wet combing found 49 cases on 224 children, whereas inspection had found only 33, of which 14 observations could not be confirmed by wet combing. Subsequent examination after 14 days confirmed 1 of the 14 as having lice, but the authors failed to explain the other 13 individuals on whom a louse was "observed" but not confirmed by their "gold standard" method of wet combing (33).

The same team investigated wet combing as a mass screening method in an area with a long history of collaboration between local school and health care organizations. They found that it was feasible but resource intensive. Each team

of 5–7 workers took 2–3 days to examine all the students in each relatively small elementary school. One worker was able to screen up to 25 students in a five-hour working day, at a cost of $10 in consumables per 25 students and a capital outlay of approximately $500 per team for permanent equipment such as wash basins and towels. However, the program was highly dependent on local community efforts and may not be easily transferable to other areas (96). A dry detection combing technique similar to that used by Mumcuoglu et al. (66) is regarded as having a high positive predictive value (84), and our laboratory team screened everyone in a school of approximately 400 students in a single school day using the method [(17); I.F. Burgess & C.M. Brown, unpublished data]. This dry-combing technique was shown to have a sensitivity of 87% [(19); C. Guzzo, personal communication].

PREDICTIVE DIAGNOSTIC VALUE OF LOUSE EGGS The presence of eggs or nits has long been regarded as putatively diagnostic of active infestation. However, this has come under scrutiny and is now no longer considered adequate evidence in the absence of living mobile stages (41). In confirmation of this, a study in which 50 children who were diagnosed as having no lice but louse eggs/eggshells within 7 mm of the scalp were followed for 14 days showed that only nine children subsequently developed an active infestation, and the authors concluded that under normal practice the remainder of the children would likely have been exposed to insecticides unnecessarily. The proximity of the eggs/nits to the scalp was a higher risk factor than the total number of eggs. Seven of 22 cases having five or more eggs close to the scalp developed an active infestation compared with only 2 of 28 that had fewer than five eggs. The conclusion was drawn that no-nit policies requiring exclusion from school may be unnecessary and that even children with eggshells close to the scalp are likely to be of limited infection risk for others (104).

Epidemiology and Control of Infestation

Relatively fewer studies of prevalence and incidence have been conducted in recent years. Older studies, mostly point prevalence surveys, have been summarized in reviews of the literature (13, 39) and newer field surveys show essentially the same patterns of infestation, although at a higher level of endemicity than had been observed in the recent past (32, 91). In contrast, a survey of public health records from 1910 to 1930 in Glasgow, United Kingdom, showed that prevalence in some communities was occasionally as high as 50% and that there was an inverse relationship between prevalence of lice on children and the tonnage produced in the ship-building yards (the main industry) in the city. Louse prevalence throughout the period was highest in communities with a low socioeconomic status (54).

The impact of diagnostic and treatment interventions on epidemiology has been poorly investigated, but a program designed to eliminate head lice from the Isle of Man, United Kingdom (population 250,000), worked for 12 months to prepare staff and the community for a louse elimination day (98). The whole community

played an important role in executing the program and parent caregivers were responsible for finding and eliminating the majority of infestations. The effect of the program, between 1986 and 1992, was that an infestation rate of 4.36% (433 cases) was initially reduced to 0.01% the following year. Voluntary notifications of infestation episodes were considerably reduced at 84, 73, 119, 82, 53, 123 in years 1–6, respectively (98). This result was achieved at a time when levels of infestation were apparently beginning to increase in other parts of the United Kingdom.

TREATMENT

The vast majority of literature on human lice relates to treatment of infestation. Since the 1940s, this has revolved around use of synthetic insecticides or synergized pyrethrum. Alternatives to insecticides have been investigated only when pharmaceutical treatments have been found less than satisfactory in their effectiveness. No alternative has yet demonstrated a greater effectiveness than insecticides when used by consumers.

It has been widely assumed that insecticides are efficient killers of insects. This assumption is commonly derived from the widespread use of these chemicals in agricultural and broader public health applications, where effectiveness beyond protecting a crop or reducing transmission of vector-borne disease is not required. However, for acceptable management of human lice 100% effectiveness is required by the consumer and such was believed to be the case for many products when they were first introduced. Whether this belief was based on the fact that they were actually effective most of the time, or whether because failures were not noticed early enough due to the poor diagnostic methods being applied is open to speculation. Various products were attributed characteristics such as a protective residual effect lasting several weeks without any clear evidence in support of the claim other than that reinfestation was not detected sooner by the methods available at the time (55, 56, 94).

Evaluation of Insecticides In Vitro

Evaluation of the activity of technical insecticides and of the activity of insecticide-based products are different entities. Methodologies for measuring susceptibility of lice to technical insecticides have long been approved by the World Health Organization, with recommended protocols and dose ranges (101–103). However, these protocols are useless for measuring the effectiveness of formulated materials, and tests that mimic consumer use of the products are required. This is the essence of the argument proposed by Burkhart & Burkhart (22), but they appear to have misunderstood the difference between determining susceptibility of an insect to an insecticide and susceptibility to that insecticide when in the presence of the excipients (components of a formulation other than the named active ingredient) in pharmaceutical products, which may enhance or inhibit activity. Measurement

of susceptibility or resistance, per se, can be performed only when using measured doses of insecticide applied via an inert substrate, and this is the basis of the WHO tests and their variants (15, 16–18, 38, 42, 50, 68, 80). Measurement of sensitivity to insecticide in formulation cannot be generic and must be measured either in vivo by means of a clinical trial or in vitro/ex vivo using the whole formulation.

Various studies have endeavored to measure the effectiveness of pediculicides in controlled laboratory test systems. On the head it is unlikely that lice or their eggs would be wholly immersed in a pediculicide fluid for more than a few seconds after which the material would disperse over the surface of hair shafts and start to evaporate. Consequently any test in which the insects are constantly wetted by the preparation, in the absence of evaporation, is likely to give a false representation of the activity. Furthermore, evidence from in vitro tests carried out in our laboratory [(15); I.F. Burgess, unpublished data] indicates that the actual application procedures are necessary to ensure a proper evaluation of effectiveness. For example, the synergized pyrethrum shampoo RID® (Bayer Corp.) applied to head lice ex vivo was found to exhibit a low activity during the period lice were wetted with the product. However, when the product was washed off after 10 min, lice immediately began to exhibit signs of intoxication (I.F. Burgess, unpublished data). Consequently, the methodology of ex vivo tests conducted by Meinking and colleagues (58, 61), in which lice were permitted to paddle around continuously on toweling wetted with pediculicide, without subsequent washing, may have given unrepresentative results in some cases (15). Nevertheless, these tests demonstrated that clear differences exist between products, when applied to a naive Panamanian population of lice not previously exposed to pediculicides (61), and that some differences had arisen as a result of reformulation (58). These studies identified 0.5% malathion alcoholic lotion, fortified with the monoterpenes *d*-limonene (dipentene) and α-terpineol, as the most effective and fast acting of the preparations and lindane shampoo as the slowest and least active, with pyrethroid products in between (58, 61). A similar result was achieved using lice from Florida, except that activity of all pyrethroid-based products was reduced, indicating a loss of sensitivity to both permethrin and pyrethrum (59).

Tests using laboratory-reared body lice may be less discriminatory than tests employing wild-caught head lice, and they do not identify variations of effectiveness likely to arise as a result of selection pressure (58). However, provided the tests mimic consumer use and do not employ absorptive substrates to which lice may cling (13, 15), they can be just as discriminatory of poor formulation (9, 10, 14, 15, 20). In such tests, malathion and carbaryl had limited and variable effectiveness, and only in formulations containing active excipients, such as high levels of monoterpenes (approximately 13%–20%), were they completely pediculicidal and ovicidal (9, 10). Pyrethroids, such as permethrin, *d*-phenothrin, and pyrethrum, are pediculicidal but of limited and variable ovicidal activity (14, 15). In this group also, formulation excipients often contribute as much to the activity as the named insecticide [(15); I.F. Burgess, unpublished data]. None of the shampoos tested demonstrated adequate effectiveness (in some cases they were

less active than cosmetic shampoos), and none was ovicidal so that even if lice were killed, the majority of products permitted them to lay sufficient eggs during the post-treatment observation period to regenerate the population (15, 20).

Evaluation of Insecticides in Clinical Trials

In many countries pediculicide products were formerly listed as toiletries or, as in the United States, were regulated by the Environmental Protection Agency. From 1970 to 1990 the majority of countries altered their regulatory procedures to classify insecticides used for treatment of human ectoparasite infestation as medicines. As a result, pediculicides are now required to undergo the same evaluation procedures as other therapeutic agents, including being subjected to clinical trials.

STRUCTURE OF TRIALS Clinical studies of the activity of pediculicides are of variable quality, and many are so old they are no longer applicable to clinical practice (35, 95). For adequate evaluation of efficacy, trials of pediculicides should not only conform to CONSORT (CONsolidated Standards of Reporting Trials) guidelines (4a) for clinical trials but also apply specific protocol guidelines. Protocols need to ensure that the methods used clearly determine whether infestation is active, that no interventions other than those being evaluated are applied, and that sufficient post-treatment observations are made that the source of any lice can be identified to provide maximum usable data. Both Vander Stichele et al. (95) and Dodd (34) made suggestions for subject-specific criteria to address the principal deficiencies found in older trials. The most important subject-specific criteria are (*a*) that infestation cannot be confirmed unless living mobile stages of lice are observed and (*b*) that the observation period post treatment should be no less than 14 days, preferably with several interim checks to monitor the course of treatment and to identify reinfestation from contacts.

Despite these criteria, no published studies conducted since have followed their recommendations completely and, apart from one published (83) and one unpublished study (I.F. Burgess & C.M. Brown), have not addressed the reasons for not following them. In addition, few studies of pediculicides comply with CONSORT guidelines for conducting randomized controlled clinical trials (4a).

Active infestation must include observation of living mobile lice rather than just louse eggs or nits. Some studies in the past have been seriously compromised by failure to find lice. For example, only eggs, many of which may not have been viable, and no lice were found prior to treatment on 73 of 77 participants in one study (46), with the result that the product under investigation was a guaranteed success regardless of how efficacious it really was.

ACTIVITY OF EXISTING PRODUCTS WITH NIT COMBING Clinical studies range from the observational to fully controlled clinical trials. Three studies have examined currently marketed products from the North American market. One trial

(223 subjects over seven interventions) compared pediculicides and the combs provided in the packs for removing nits. It examined five synergized pyrethrum products, which conformed to the FDA Monograph on pediculicides (38a), 1% permethrin crème rinse, and 1% lindane shampoo and found none of them to be wholly effective. At that time the combination of permethrin with its package comb was marginally more effective and there were differences in effectiveness of Monograph products, despite their apparent similarity, and lindane was considered the least effective (30). However, a subsequent comparison of one of those synergized pyrethrum products and its comb (RID) with 1% permethrin crème rinse and its comb (NIX® Warner Lambert) found both to be wholly effective (4).

A more recent evaluation compared the efficacy of 1% permethrin with or without combing. It found 83.1% of participants in the group treated only with permethrin (95% CI, 71.0–91.6) were louse-free one day after treatment, After one week, before a second application of treatment, only 45.8% (95% CI, 32.7–59.2) remained louse-free. However, the proportion without lice rose to 77.6% (95% CI, 64.7–87.5) immediately after retreatment, and by day 15, 78.3% (95% CI, 65.8–87.9) had no active infestation. At all four assessments the level of infestation was higher in the group receiving combing as well as insecticide, showing that use of a nit comb did not reduce the risk of continued infestation and could not be relied upon to remove all the eggs and nits (57). In this study, it is likely that resistance to permethrin played a role in treatment failure, but the inability of the investigators to eliminate infestation by combing confirms anecdotal information that combing procedures and no-nit policies have not assisted caregivers in eliminating infestation from their children.

NEW ACTIVE SUBSTANCES Some studies have been conducted to look at potential new active substances for use against head lice. After resistance to pyrethroids was identified in the Czech Republic in 1992, alternatives were sought on the basis of data obtained from in vitro studies conducted a decade earlier (86, 87). This resulted in the introduction of new lotion products containing 0.3% malathion or 0.5% pirimiphos-methyl and a shampoo with 0.7% pirimiphos-methyl. Community trials of these products were claimed to provide 100% effectiveness (87).

A similarly uncontrolled trial of topically applied ivermectin was conducted in Egypt using 25 subjects who received a single application of 0.8% ivermectin in a liquid vehicle. The preparation was found to kill all lice and their eggs (107). The rationale for this work was based on veterinary data in which ivermectin was used to control ectoparasites on livestock and experimental work in which human lice fed ivermectin-treated blood in the laboratory were killed (70). Membrane-fed lice were susceptible to dose levels of 10 ng ml^{-1} or less, with an LD_{50} between 1.25 and 2.5 ng ml^{-1} and an LD_{95} between 5 and 10 ng ml^{-1} for both nymphs and adult females. Rabbits injected with 200 μg kg^{-1} proved toxic to lice for 2 or 3 days, after which activity declined (70). However, it is possible that this effect was slightly enhanced because laboratory-reared lice take relatively large blood meals, which would increase the amount of ivermectin ingested, whereas head

lice, which take only small amounts of blood at one time (47), may not be exposed to such high doses via their food.

Interest in ingested pediculicides has existed since Shashindran et al. (90) chanced to observe that the antibiotic co-trimoxazole (trimethoprim/sulfamethoxazole, TMP/SMX) has a lethal effect on mobile stages of lice, apparently because it kills the symbiotic microorganisms living in the mycetome of the louse. A single randomized study conducted in the United States investigated this effect in comparison with 1% permethrin crème rinse and with a combination of the two therapies (43). The treatment regime with TMP/SMX was 10 mg kg^{-1} day^{-1} over 10 days. Permethrin crème rinse was applied once for 10 min, with a follow-up application after one week if required, and combination therapy comprised both regimes at the same time. Fourteen days after initiation of treatment, 11 of 39 participants treated with permethrin still had lice compared with 8 of 36 treated with TMP/SMX and 3 of 40 treated with the combination. However, the weaknesses of this study are that caregivers made nearly half the evaluations and that the information was obtained by telephone interviews. Furthermore, the assertion by the authors that TMP/SMX is a familiar drug that would be more appealing to parents for dealing with refractory cases of infestation is not convincing, especially because five participants developed adverse reactions of nausea, vomiting, and/or rash, nine experienced intense but transient pruritus, and the treatment of three participants was terminated early because of an allergic-type reaction (43).

COMBING AS A TREATMENT Combing options for treatment have gained in popularity in recent years, partly out of concern about the toxicity of pesticides (1a, 88, 106), partly as a way of dealing with infestations resistant to conventional insecticides, and partly as a way of reducing the cost of treatment (44). Various organizations have their own preferred methods (2, 31, 73), but as yet none has produced convincing clinical evidence for their proposals. Indeed, both past and recent history have indicated that combing as the only means of treatment (6, 40, 79, 83) or as an adjunctive therapy (57) is limited in its effectiveness and may not be as popular with caregivers as some people might suggest. An observational survey of Belgian children diagnosed by investigators and then treated at home found that 47% (18 of 38) of parents who opted to wet comb, using conditioning crème rinse as a lubricant, were successful compared with 56% (14 of 25) who chose to use insecticides. A combination of the two methods proved less effective with only 37% (7 of 19) being cleared of lice (96).

Comparisons of insecticide and wet combing treatments, using a commercially available combing pack under the name of "Bug buster," have been made in four United Kingdom–based randomized trials of varying quality.

A pilot study, in which participants attended a treatment clinic, compared a single application of 1% permethrin crème rinse with four wet-combing sessions applied over two weeks (6). A researcher applied lotion or the first session of combing and parent caregivers applied subsequent combing sessions. The result was inconclusive due to a high drop-out rate of 40% (10 of 25 participants). Of the

15 who completed the study, three of seven participants treated with permethrin and six of eight treated by combing were observed to be louse-free at the end of the study period.

A second small study (30 children) comparing two applications of 0.2% *d*-phenothrin lotion seven days apart with wet combing over two weeks reported that 2 of 15 treated with insecticide and 8 of 15 who were combed were louse-free after 14 days (79).

A medium-sized, community-based study (74 of 81 participants completing the study) compared the effectiveness of two applications of 0.5% malathion products seven days apart (27 cases using alcoholic vehicle containing 13% terpenoids and 13 cases using aqueous emulsion) with that of four sessions of wet combing over 14 days. The cure rate was 78% (31of 40) for the insecticide group but only 38% (12 of 32) for the combing group. Those using wet combing were 2.8 times (95% CI 1.5–5.2) more likely to have lice after treatment than those treated with malathion ($p = 0.0006$) (83).

A large study in the community (275 of 278 participants completing the study) compared single applications of either 0.5% *d*-phenothrin mousse or 0.2% *d*-phenothrin alcoholic lotion with four sessions of wet combing over 14 days (I.F. Burgess & C.M. Brown, unpublished data). The cure rate showed no significant difference between treatment methods at 20% (21 of 105) for *d*-phenothrin mousse, 28% (30 of 107) for *d*-phenothrin lotion, and 22% (14 of 63) for wet combing. People receiving mousse were 1.4 (95% CI, 0.9 to 2.3) times more likely, and those being wet combed were 1.26 (95% CI, 0.7 to 2.2) times more likely, to have lice after treatment than those treated with lotion. This study employed more intensive follow-up of participants in line with the recommendations set out by Dodd (34), and interpretation of the ages of lice found showed that a repeat insecticide application after seven days could have increased effectiveness to 58% for the *d*-phenothrin lotion and 30% for the mousse (19).

Insecticide Resistance

Most insecticides employed against human lice have been used in consumer products for decades. Although it was long recognized that resistance to most insecticides would develop eventually, public health authorities and the pharmaceutical industry have been slow to respond to resistance, as now it is a reality.

CLINICAL EVALUATION Pyrethroid insecticides are the most widely used for control of head louse infestation, with products based on natural pyrethrum, permethrin, *d*-phenothrin, bioallethrin, or tetramethrin. One clinical study has addressed the problem of insecticide resistance coupled with in vitro and ex vivo laboratory tests. Following reports of failed treatments in France, a comparison was made of single applications of 0.5% malathion lotion or 0.3% *d*-phenothrin lotion (28). In the clinical phase of the study 87 of 95 (92%) participants treated with malathion were louse-free one day after treatment, but this increased to 91of 95 (95%) by

day 7. In contrast, of those treated with *d*-phenothrin only 39 of 98 (40%) participants had no lice one day after treatment, decreasing to 38 of 98 (39%) by day 7. Five of the children in the malathion group and 27 in the *d*-phenothrin group changed their status from louse-free to infested, or vice versa, between days 1 and 7 without further intervention from the investigators. This suggests that examinations so soon after treatment provide relatively little useful information on treatment success and also that, despite admonitions to the contrary, some parental caregivers must have engaged in some additional intervention such as nit-combing to remove lice. Coupled with the clinical evaluation, lice removed from some children were tested for sensitivity to the two preparations ex vivo by a modified form of a WHO method for evaluation of insecticide susceptibility (28, 103). All lice exposed to the malathion product were killed in 60 min, whereas 82 of 416 (19.7%) of those exposed to *d*-phenothrin were still alive after 24 h, 6% more than in the untreated control group, in which 44 of 320 survived.

LABORATORY EVALUATIONS Resistance to insecticides has been identified only by ex vivo testing. The majority of studies have used WHO-recommended protocols (102, 103) or modified versions of those techniques. Resistance to permethrin and related pyrethroids, such as *d*-phenothrin, bioallethrin, and β-cypermethrin, has been identified in several studies using lice taken from people who had suffered treatment failures when using products that had apparently been effective a few years earlier. Failure of *d*-phenothrin due to resistance was first properly documented in France in 1992 (28). Concurrently, studies were under way in other countries where permethrin was more widely employed and found to have lost its effectiveness. A study in the Czech Republic employed a rather different methodology from all other studies by application of different concentrations of insecticide solution to tresses of simulated hair (monofilament nylon) (87). This study found considerable loss of sensitivity to permethrin in head lice from various parts of the country, compared with the level of sensitivity observed 11 years earlier, with resistance factors ranging from 5 to 577 at LC_{90} (86, 87). Nevertheless, the dose concentrations of insecticide used for these tests were low compared with the levels in commercial preparations; for example, in Prague the LC_{50} and LC_{90} were only 0.0044% and 0.1214%, respectively, in the solutions applied to the nylon hairs.

Two studies evaluated resistance to permethrin using techniques on the basis of a WHO method employing single concentration applications of insecticide to filter papers, and measuring time to death of the test insects, in order to record LT_{50} and LT_{95} (103). In the United Kingdom, lice were constantly exposed to the insecticide, other than when they were being fed. Papers were treated with 0.1% (6.3 μg cm^{-2}) and 0.25% (15.8 μg cm^{-2}) permethrin in propan-2-ol, which was then evaporated. Permethrin failed to kill between 27 and 48% of lice over more than 48 h (18). No data were available from before the introduction of permethrin to the United Kingdom, but it was estimated that these lice showed approximately 16-fold greater resistance compared with levels measured in lice from Israel (69). Increasing the level of permethrin to 1% (63 μg cm^{-2}) failed to increase mortality,

and by 1999 the majority of louse samples tested from around the United Kingdom were resistant to permethrin and/or malathion and some had acquired resistance to carbaryl (16, 36, 38). However, in one of these studies the levels of insecticide used to measure the lethal dose were considerably lower than those recommended by WHO (101), so in some cases the measure may have been more of vigor tolerance (reduced sensitivity of an insect population due to elimination of the least robust insects by weak selection) than resistance (38).

Resistance to these insecticides has been identified as having developed through multiple biochemical pathways, including nerve insensitivity (knockdown resistance) for pyrethroids, specific and nonspecific esterases for pyrethroids and malathion, and selection of alternative choline esterases for malathion and carbaryl [(6, 50); I.F. Burgess, unpublished data]. These investigations led to the development of a simple test kit for community health workers that could also be used in the field to evaluate resistance to several insecticides (17).

A similar methodology was used in Israel to evaluate resistance to permethrin (68). In this study also, the level of permethrin on the test papers, using a relatively nonvolatile silicone fluid (Dow Corning 556) as a solvent vehicle, was increased from 0.25% to 1% in an attempt to decrease the time required to achieve over 90% mortality in the lice; however, no significant decrease was achieved. These lice exhibited 4.1-fold greater resistance compared with the sensitivity measured five years previous (68, 69). An attempt to elucidate the mechanism of action found a pre-existing glutathione S-transferase-based mechanism of DDT resistance that predated the introduction of permethrin, no esterase activity, and only weak monooxygenase activity. The resistance identified was likely due to a combination of knockdown resistance–like nerve insensitivity and monooxygenase mechanisms (42).

In the Americas, resistance to pyrethroids was first confirmed in Argentina, employing a range of concentrations of insecticides (permethrin, *d*-phenothrin, and deltamethrin) in dioctyl phthalate to measure LC_{50} only (77, 78). Lice were exposed for only 1 h and were then transferred to clean filter papers to be evaluated after 18 h. The resistance ratio, compared with susceptible lice, ranged from approximately 16 times to approximately 90 times with LC_{50} values ranging from 1.02% permethrin, for susceptible lice, to more than 90% permethrin, with the greatest resistance found in communities with the highest socioeconomic status. No resistance was found to carbaryl (77). Inhibition of monooxygenase by means of piperonyl butoxide increased susceptibility in lice from several communities. Inhibition of specific carboxylesterases using triphenyl phosphate increased susceptibility in the same groups of lice but to a lesser degree (78). It is likely that sensitivity of lice may have appeared lower in this study than was actually the case because experiments in our laboratory have found that lice take up insecticides more slowly from dioctyl phthalate, the solvent vehicle used, than from some other solvents (I.F. Burgess, unpublished data).

In the United States, permethrin resistance was confirmed first in Massachusetts and Idaho in trials in which they were compared with susceptible lice from Sabah

in Borneo (80). In this study, various concentrations of permethrin were dissolved in acetone. American lice were exposed to dry deposits of insecticide for 18 h, whereas those from Borneo were evaluated after just 6 h because control mortality increased in this population from 8 h after removal from their hosts. About half the lice from Borneo exposed to deposits from 0.03% solutions were immobilized, as were nearly all those exposed to residues from 0.3% solutions. Lice from the United States were not affected by these concentrations (80).

A second U.S. study evaluated the effects of dry deposits from 0.5%, 1%, and 10% acetone solutions of permethrin on lice from Florida and Massachusetts in comparison with laboratory-reared body lice, head lice from Panama, and head lice from Bristol, United Kingdom (50). This study was different from all others in that it evaluated the sensitivity of newly emerged and fed first-instar nymphs and measured LT_{50} relative to body weight. This technique has previously been applied to relatively large insects such as Colorado potato beetles (49) but has disadvantages when applied to lice because they take variable-sized blood meals and after feeding constantly lose weight in the form of water. The results obtained showed considerably longer times than previous studies to achieve LT_{50}, at $\sim$700 min for laboratory-reared lice exposed to 0.5% permethrin-treated papers compared with 55 min (69) and 59 min (18) for laboratory-reared lice exposed to 0.1% permethrin-treated papers. Effects of resistance on knockdown time were determined in addition to the effects on mortality. A low level of monooxygenase activity was found in Massachusetts and Panama lice but not in Florida lice.

These investigators also cloned the *para*-orthologous sodium channel α-subunit cDNA fragments spanning the $\sim$IIS4-IIS6 region. From the amino acid sequences they were able to identify a sodium channel mutation, similar to one previously found in diamondback moths, and a second mutation, not previously identified, in lice from both Florida and Bristol (50). This knockdown resistance–type mutation was not identified in lice from Panama and not confirmed in Massachusetts lice, in which oxidative mechanisms appeared as the most important identified component of resistance.

Alternative Treatments and Active Excipients

In the absence of effectiveness of both conventional insecticides and mechanical methods for removing lice, consumers and investigators are now seeking new methods for eliminating louse infestations. The activity of excipient chemicals in pediculicide formulations is both little understood and poorly investigated.

SOLVENTS Organic solvents inevitably exert some activity on the insects and alcohols have long been used as solvents for delivering insecticides in hair preparations. Shorter-chain alcohols such as ethanol and propan-2-ol (isopropanol, or isopropyl alcohol) have relatively little activity unless used undiluted. However, longer-chain alcohols are toxic to lice at relatively low concentrations. Primary alcohols with carbon chain lengths ranging from 8 to 12 exhibit activity, increasing

with the length of the carbon chain (62). The highest activity, from 1-dodecanol (LC_{50} 2.28% in 60% isopropanol) showed knockdown of permethrin-resistant lice in 10 min and mortality after 18 h. The lowest activity was from 1-octanol (LC_{50} 4.46%) when mixed with 60% isopropanol. If long-chain alcohols were diluted with water, they induced knockdown but the lice recovered within 18 h. Addition of 5% or 10% 1-dodecanol to 0.2% *d*-phenothrin lotions enabled the product to kill more than 95% of permethrin-resistant lice in vitro (63).

NIT REMOVERS Although products claiming to facilitate removal of nits and louse eggs from hairs have been sold for some time, there is no scientific or clinical evidence that they speed the nit removal process or make it less uncomfortable. The one published clinical study of a nit removal product was seriously flawed because the comb used in conjunction with the product was metal and thus more efficient that the plastic combs used in conjunction with the other products used for comparison (32a). Having knowledge of the biochemical structure of the cement that lice use to hold the eggs in position is the first step toward developing a product of this type that actually works. A single study examined this material using flash pyrolysis-gas chromatography/mass spectrometry and it found that the material is primarily amino acid based with some fatty acids present (23). Several of the components are analogous to components in human hair, making a biochemical method for disrupting louse egg glue quite difficult to pursue.

BOTANICAL EXTRACTS Monoterpenoids incorporated into conventional insecticide products are active against both lice and their eggs (9, 10), so further investigation of their activity became a natural first point for investigation of potential new materials for use against lice. The principal source of monoterpenoids is plant essential oils, many of which have been considered to have insecticidal activity by herbalists and others in previous centuries, with the result that some of the source plants have been given trivial names that reflect their supposed applications such as "lousebane" and "fleabane." Others such as juniper, eucalyptus, lavender, geranium, lemon, and rosemary have also been reputed to have insecticidal activity (105). Practitioners of aromatherapy use whole essential oils in low concentrations (usually a few drops in 100 ml carrier fixed vegetable oil) to avoid the risk of human toxicity. In some communities the idea that a natural substance could be used to kill lice has proved popular, and numerous nonmedicinal preparations that contain dose levels that are probably harmless to humans (i.e., many contain plant extracts at concentrations lower than those used in traditional medicines) have been developed. None of these materials have been submitted for appropriate evaluations of efficacy or toxicology by regulatory authorities and are often marketed as repellents, combing aids, or preparations to improve scalp health. Despite the claims, there is no published evidence that any of these products are pediculicidal or ovicidal at the concentrations employed, although most manufacturers claim testimonials from consumers. Some of the products tested so far in

our laboratory show less activity than toiletry shampoos and crème rinses (E.R. Kidman, unpublished data).

Investigations of specific essential oil activity in the laboratory have looked at single oils, mixtures of oils, and individual component monoterpenoids. An investigation of mixtures of oils found that mixtures of peppermint (*Mentha piperita*) and nutmeg (*Myristica fragrans*) in a ratio of 3:7, and tea tree (*Melaleuca alternifolia*) and cinnamon leaf (*Cinnamomum zeylanicum*) in a ratio of 1:1, were effective against lice when used at a concentration of 1% dissolved in 40% ethanol (97). The same mixtures also showed some activity against louse eggs when left in contact overnight and then washed off with an acidified rinse containing 0.1% of the appropriate oil mix. Individual essential oils also varied in their activity. Seven oils with the same concentration and solvent mix were investigated. Of these, only aniseed (*Pimpinella anisum*) and oregano (*Origanum vulgare*) showed a high level of activity against lice and their eggs in both alcoholic and aqueous vehicles. Oils of rosemary, pine, tea tree, cinnamon leaf, and red thyme showed variable activity, but in all cases an acidified rinse containing low levels of the oils enhanced activity (97).

Following this work, a product based on oil of aniseed, together with coconut and ylang ylang oils, was tested clinically in a randomized trial in Israel using two applications 10 days apart. In comparison with a spray containing malathion, permethrin, and piperonyl butoxide, the anise-based product successfully treated 60 of 65 participants (92.3%) and the insecticide-based comparator product 59 of 64 (92.2%) (71).

Other workers have looked at individual terpenoids and have shown that components of cypress oil (*Cupressus* x *leylandii*) such as terpineol, camphene, and α-pinene were more active than the whole oil (99). There is a structure-activity relationship of terpenoids and those effective against lice may be different from those showing optimal activity against louse eggs [(82); C.M. Priestley, I.F. Burgess & E.M. Williamson, unpublished data]. A small ex vivo study of tea tree oil and some of its components found they were only partially active against head lice (37). It is possible that the low sensitivity was due to previous exposure to monoterpenoids in so-called aromatherapy preparations. Observations of lice that survived adequate clinical applications of insecticide products containing more than 13% monoterpenes suggest they have already become resistant to these chemicals (I.F. Burgess, unpublished data).

REPELLENTS Interest has also been expressed in repellents to deter lice from climbing onto a new host. They can be effective only if prevalence of infestation is low and only if the recipient of treatment has no lice initially (12). One substance with repellent activity identified in vitro, with a filter paper arena treated with 42 μg cm^{-2}, was piperonal, also known as heliotropine (11). This material was subsequently commercialized as a 2% spray application, but a double-blind, cross-over, clinical field trial, in which more than 40 families participated for over 6 months, failed to demonstrate clear effectiveness (C.M. Brown & I.F. Burgess, unpublished

data). The relative activity of various essential oils and terpenoids applied to hair substrates in vitro exceeded that of the mosquito repellent N,N-diethyl-3-methylbenzamide (formerly known as N,N-diethyl-m-toluamide, or DEET) (67). Apart from citronella oil and its active component citronellal, none gave more than 50% repellency, even when used undiluted, or was active for more than a few hours owing to the volatile nature of the compounds.

HOST IMMUNIZATION Immunization of the host against lice may be possible, as has been achieved with some other pest species (1, 45b). The aim is to target sites on the surface of gut cells and either kill the lice or limit fertility and fecundity. Rabbits, used to feed a laboratory body louse colony, were immunized with three doses of 500 μg protein extracted from louse midguts. Lice fed on immunized rabbits had higher mortality, with more frequent rupture of the gut, than those fed on control rabbits. The lice also took smaller blood meals and laid fewer eggs and nymphs took longer to develop (5). Analysis by SDS-PAGE electrophoresis found 18 proteins between 12 and 117 kDa in the midgut extract used for this immunization. Antibodies from immunized rabbits reacted with all proteins in the extract (75) and similar results were obtained using polyclonal sera from rabbits immunized against other species of lice (*Haematopinus africanus* and *Linognathus stenopsis*) or louse feces, all of which contained four common antigens (64). When lice fed on rabbits immunized with fecal extracts, they ingested significantly less blood and laid fewer eggs, which were less viable, and the emerging nymphs took longer to develop. However, the survival rate of lice fed on immunized rabbits was no different from the survival rate in the control group (65). By means of immunofluorescence and immunogold techniques, immunogenic antigens were located mostly on microvilli of gut epithelial cells, with some in the lumen of the gut (72).

FUTURE PROSPECTS AND NEEDS

Prevalence of head louse infestation is increasing worldwide because of the impact of insecticide resistance. To date, no viable alternative therapy to replace insecticides has been developed and, in the absence of either willingness or skill on the part of parent caregivers to remove all lice by physical means, the need to find a replacement therapeutic material is paramount. In some communities in Britain it now appears that some families have become resigned to the inevitability of lousiness during childhood and are making efforts only to manage the number of insects on the children's heads (C.M. Brown, unpublished data).

Although no data are available, there are anecdotal indications that body louse prevalence is also increasing, albeit much more slowly. This is a more serious health threat due to this louse's ability to act as a disease vector. In the recent past the risks of typhus (*Rickettsia prowazekii*) outbreaks have been diminished and none occurred following either the 1990s wars in the Balkans or the 1991–1992

Gulf Conflict, in which large numbers of displaced people suffered varying states of lousiness. However, some outbreaks have occurred in Burundi (7) and louse-borne relapsing fever (*Borrelia recurrentis*) is still common in parts of eastern and central Africa, especially among poor and displaced people in Ethiopia (93), and could spread with refugees from any future conflict.

The emerging louse-borne infection is quintana (trench) fever (*Bartonella quintana*), which was largely thought to have disappeared after the World Wars (85). This organism has recently been identified by polymerase chain reaction from lice or culture from lice or blood taken from homeless people in Paris, Seattle, and Tokyo (8, 48, 89). If economic factors result in a further increase of body louse prevalence, it is certain that this infection will increase also.

The priorities for future advances in louse management have changed since I last wrote on this subject in 1994 (13). Regulatory and public health authorities, the pharmaceutical industry, and the public have all been slow to respond to control pressures generated by the appearance of insecticide resistance. Even if new treatments are introduced for control of head lice, they will not be as effective as people wish unless our understanding of lice is improved, including improving diagnostic methods. Most important is improving our knowledge of fundamental louse biology and physiology, areas where current misunderstanding and lack of knowledge result in a great waste of financial and time resources by professional and family caregivers alike. Failure to learn from the past is likely to limit not only the effectiveness of any new control measures but also the viable life of these measures, as pressures for lice to develop new forms of resistance arise.

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