recording. The song of this insect has a frequency spectrum of about 7–80 kHz.

The songs of *C. parallelus* and *M. brachyptera* were next recorded at 37.5 i.p.s. (95 cm/sec) through a condenser microphone and played back to the preparation through the same microphone. In this way recording and reproduction of song elements with frequencies up to at least 150 kHz was achieved. Stimulation with a wide frequency band was not always effective as the preparation was found to be highly sensitive to any high frequency background noise generated by reproduction, and the response to this swamped responses to the song. When the song was played back through electronic filters set to give a narrow frequency band of emission with slopes of 24 dB/octave either side of selected frequencies, responses comparable with those obtaining from the song of a caged insect were elicited within the range of centre frequencies from 35–100 kHz. Above this frequency the filters were relatively ineffective, while below 25 kHz the signal to noise ratio of the nervous response was greatly diminished.

The high frequency transients in the songs were identified by monitoring with a tuned ultrasonic detector operating over a 5 kHz band. A direct comparison was possible between these records and the sequence of action potentials on the nerve (Figure 2). The relationship between the two was assessed by counting the number of large ultrasonic 'spikes' in a sound pulse and comparing these with the number of action potentials over an arbitrarily chosen but consistent height. The results are shown in the Table.

Correlations between ultrasonic 'spikes' in song pulses and numbers of large action potentials

Stimulus		Corr. coefficient	No. of pulses
Chorthippus song,	caged insect	0.97	15
	audio tape recording	0.74	24
	tape recording at 25 kHz	0.98	7
Metrioptera song,	caged insect	0.75	27
	audio tape recording	0.03	14
	tape recording at 65 kHz	0.73	18

It is evident from the correlations and from visual inspection of high speed oscillograms that at frequencies within the range of the species song the tympanal organ responds with a high signal to noise ratio and that the response reflects the number of transients in the song pulse. At audio-frequencies, on the other hand, the organ responds with a low signal to noise ratio; the response is relatively disordered and reflects the approximate duration of the pulse (or amplitude modulation envelope) but not its transient structure.

It is clearly essential, in physiological or behavioural experiments on insect hearing, to ensure that the transient characteristics of the species song are adequately produced at the appropriate frequencies, otherwise the results may have little biological significance. The theory that the nervous response follows the amplitude modulation envelope should now be disregarded, for our evidence indicates that the response normally mirrors the succession of transients within the natural song pulse.

Résumé. Les réponses du nerf tympanique des Tettigonides sont conformes à la structure actuelle des transmetteurs du chant naturel mais non pas si le chant est reproduit par enregistrement magnétique ordinaire. On a montré que les constituants ultrasoniques sont essentiels et que les réponses parfaites ne sont obtenues que lorsqu'on a pu produire des fréquences très hautes.

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The Antimicrobial Activity of Citral

The antimicrobial activity of essential oils has been the subject of numerous publications, and many of the constituents of these oils have been tested for activity. In particular, citral has been cited by many authors as having substantial antimicrobial activity. BALAKHOVSKIĬ and MEĬSEL[1] in 1945 reported on some fragmentation products of vitamin A, one of which was citral. They stated that citral had antimicrobial activity and suggested some possible uses in clinical medicine. The activity of citral isolated from lemon grass oil against *Bacillus typhosus* was reported by BOSE et al.[2], in 1949. Of the compounds tested, citral was the most effective, having a Rideal-Walker coefficient[3] of 20.0. The same authors[4] stated in 1950 that the germicidal power of lemon grass oil was directly proportional to the citral content. MASHIMO, SERISAWA, and KURODA[5] have shown that citral strongly inhibited *Micrococcus pyogenes* var. *aureus* but was ineffective against *Salmonella enteritidis* and *Pseudomonas aeruginosa*. Likewise, OKAZAKI and OSHIMA[6] reported that citral (along with other compounds) was effective against fungi. They tested *Epidermophyton inguinale, Achorion gypseum,* and *Trichophyton interdigitale*. It was fairly active against *Mycobacterium tuberculosis* (avian type) but ineffective against *Escherchia coli, B. dysenteriae,* and *Staphylococcus aureus*.

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In a search for antimolding agents for syrups, LORD and HUSA[7] found that 1 sample of citral was effective against molds growing on syrup at a dilution factor between 1:2000 and 1:5000. Another sample of citral was found to be effective between 1:5000 and 1:10,000. KELLNER and KOBER[8,9] also suggested that citral could be used as a room disinfectant. They found it to be fairly active against *Neisseria, Streptococcus faecalis, S. aureus, B. megatherium,* and *Oidium albicans*. Citral was less effective in controlling *E. coli, Eberthella typhosa, S. pyrogenes,* and *Corynebacterium diphtheriae*.

An extensive examination was made by MÖSE and LUKAS[10] in 1957 into the antibacterial action of some essential oils. They tested citral, among other compounds, against 34 species and strains of bacteria. Of the compounds tested, citral was found to be the most active, being particularly potent against 14 of the bacteria tested.

More recently, ZIBITSKER[11] in 1960 tested citral against 12 strains of *Candida*. Fungistatic activity was found against 7 of the *Candida* strains at serial dilutions of 10^{-6} to 10^{-8}. Fungicidal activity was found at dilutions of 10^{-4} to 10^{-7}. MARUZELLA et al.[12] found that the vapors of citral (along with several other compounds) were active against *C. albicans, Phoma betae, Geotrichum candidum,* and *Oospora lactis*.

In general, citral in various and undefined states of purity was found to be fairly active against fungi and less active against bacteria although it does show strong action against some strains.

In our studies of the antimicrobial activity of essential oils and, in particular, of those compounds which are responsible for the activity, we had occasion to re-examine the activity of citral. Citral (Fritzsche Bros., rectified) was purified by formation of the bisulfite addition

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Table I. Activity of citral against bacteria

Compound	*Bacillus cereus*	*Sarcina lutea*	*Staphylococcus aureus*	*Streptococcus elactis*	*Acaligenes faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Serratia marcescens*	Conditions ppm	h
Citral	+	+	+	+	+	—	—	—	—	500	48
Citral	+	±	±	±	+	±		±	±	250	48
Geranial	—	—	—	0	—	—	—	—	—	500	48
Neral	—	—	—	—	—	—	—	—	—	500	48
Neral/Geranial 50:50	—	—	—	0	—	—	—	—	—	500	48

+, total inhibition; —, no inhibition; ±, slight growth of microbe; 0, control did not grow.

Table II. Activity of citral against yeast

Compound	*Zygosaccharomyces japonicus*	*Candida tropicalis*	*Pichia chodati*	*Hansenula anomala*	*Saccharomyces cerevisiae*	Torula	Conditions ppm	h
Citral	+	+	+	+	+	+	250	48
Citral	+	+	±	±	±	±	62.5	48
Geranial	+	+	+	+	+	+	250	48
Neral	+	+	±	±	+	±	250	48
Neral/Geranial 50:50	+	+	+	+	+	+	250	48

Table III. Activity of citral against mold

Compound	*Aspergillus oryzae*	*Aspergillus niger*	*Penicillium chrysogenum*	*Rhizopus senti*	*Botrytis cinerea*	*Byssochlamys fulva*	Alternaria	Conditions ppm	h
Citral	+	+	+	+	+	+	+	500	96
Citral	±	±	+	—	+	+	+	125	96
Geranial	—	—	—	—	+	+	+	500	96
Neral	—	—	—	+	+	+	+	500	96
Neral/Geranial 50:50	—	—	+	—	+	+	+	500	96

compound, recrystallization, regeneration of the citral, and distillation at reduced pressure. The purified material was then tested against bacteria, yeasts, and molds (Tables I, II, and III).

The purified citral shows activity against all the gram-positive bacteria at 500 ppm[13] and one of the gram-negative bacteria. The effect drops off somewhat at 250 ppm as indicated in Table I. However, when one separates the citral into its components (neral and geranial, geometric isomers) by preparative gas-liquid chromatography and tests these under the same conditions, both are essentially inactive against all of the bacteria tested. To eliminate the possibility of a synergistic effect, a 50:50 mixture of neral and geranial was prepared and tested under identical conditions. The results were the same as those obtained with the pure components.

A similar experiment was conducted with representative strains of yeasts and molds and the same pattern emerged (Tables II and III) but the results were not quite as dramatic.

The activity of citral against the species of yeasts does not begin to drop off until a concentration of 62.5 ppm is reached. In contrast, neral begins to be ineffective at 250 ppm. It is of interest to note that geranial is somewhat different than neral since total inhibition of yeast growth is observed at 250 ppm. There does not appear to be any difference between geranial and neral with respect to bacteria and molds.

The effect of concentration of citral against various species of molds is summarized in Table III. At 125 ppm citral begins to lose effectiveness against *Aspergillus oryzae*, *A. niger*, and *Rhizopus senti*. At 125 ppm, it is still effective against the 4 remaining molds. Geranial was found to be ineffective at 500 ppm against 4 of the 7 molds, whereas neral was ineffective against 3 of the 7 molds. It becomes quite obvious that an impurity exists in the 'pure' citral.

An interesting observation is found in the action of a 50:50 mixture of neral and geranial against *Penicillium chrysogenum*. Neither neral nor geranial alone at 500 ppm was effective against this species of mold; yet, a combination of 250 ppm of each showed activity. It appears that this is one instance of synergism. Several examples of synergistic effects are reported, e.g., ZIBITSKER[11].

These data, then, cast doubt on the antimicrobial activity of citral as reported in the literature. The 'pure' citral used in these experiments was as pure or purer than that used by the previous workers. As can be seen, the 'pure' citral showed considerable activity but the isolated components failed to substantiate the previous findings.

In view of these data one must conclude that an impurity exists in the 'pure' citral which has substantial activity and which also is carried through the purification process. An examination of an analytical gas chromatogram of the purified citral showed it to be approximately 98% citral. Work is now in progress to identify the impurities and determine which of these have antimicrobial activity.

Zusammenfassung. Bestätigung früherer Feststellungen, dass Citral eine antibakterielle Aktivität besitzt. Da Neral und Geranial (allein oder zusammen) jedoch wesentlich weniger aktiv sind, muss für die Citralaktivität eine bisher nicht identifizierte Substanz verantwortlich sein.

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Macrophage Activity of Thymectomized Mice Infected with *Leishmania donovani*

Although the importance of the thymus in cell-mediated immunity and in the production of some humoral antibodies is established[1], there are several contradictory reports on the activity of macrophages in thymectomized animals[2–6].

In mice experimentally infected with the intracellular protozoan parasite of macrophages, *Leishmania donovani*, the number of parasites in the liver and spleen reach a peak after which numbers of parasites in these organs decline and the infection becomes chronic[7]. Thus, this infection in mice could be a good system to determine the effect of thymectomy on macrophage activity in vivo and in vitro.

Balb/c mice, thymectomized[8] or sham-thymectomized within 24 h of birth, were used in the in vivo studies. Each mouse was injected i.v. with 10^7 amastigotes[9] of the 3K strain of *L. donovani*[10] in 0.2 ml of infected hamster spleen suspension 25–30 days following thymectomy. Mice from each group were sacrificed 1 h after infection to determine the initial uptake of parasites by spleen and liver, and at 1, 8, 16 and 21 days to determine rate of growth or suppression. The median level of infection in livers and spleens of 5–8 mice of each group was determined by a method of touch preparation[10]. At necropsy, thymectomized mice were examined macroscopically and microscopically to determine whether thymectomy was complete. Mice with remains of thymus were not included in the results.

Peritoneal macrophages from 25- to 30-day-old thymectomized, sham thymectomized and intact mice were

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