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THE ANATOMICAL AND MECHANICAL BASIS OF STIMULATION AND FREQUENCY ANALYSIS IN THE LOCUST EAR

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SUMMARY

This paper re-examines the physical basis of frequency analysis and adequate stimulation of the receptors of the locust ear.

The anatomy of the locust ear was examined by dissection, serial sections and SEM. The compliance of the tympanum and Müller's organ were measured both with a force transducer and by application of known air pressure. The motion of the tympanum and of Müller's organ excited by a calibrated closed sound field was measured under stroboscopic illumination. This allowed the relative phase between the driving force and resultant motion, and the amplitude of motion, to be measured simultaneously.

The mass of the tympanum is irregularly distributed. Laterally, there is a thick region with endocuticle. Mesially, the sclerotized folded body and styliform foot are surrounded by regions of mesocuticle about 3 μm thick, but separated from the mesial rim of the membrane by an arc less than 1 μm thick and from each other by an area 1·4 μm thick which surrounds the pyriform vesicle.

The different sclerites and their surrounds have different compliances and masses. For the folded body region these are 0·38 m.N^{-1} and 3·9 μg, for the styliform body 0·21 m.N^{-1} and 2·9 μg and for the pyriform vesicle 0·22 m.N^{-1} and 0·32 μg. The calculated resonant frequencies of these regions are: folded body 3·5–4·1 kHz, styliform body 5·5–6·5 kHz and pyriform vesicle 16–19 kHz. Müller's organ has a mass of 10 μg, a compliance, with respect to the tympanum, of about 7·5 m.N^{-1} and a calculated resonant frequency of 0·58 kHz. The 'thick membrane' is more compliant than the 'thin membrane' and appears to act as a hinge between the thin membrane and the lateral edge of the ear.

When driven from one side by sound pressure, the tympanum vibrates. Resonances were seen, on the folded body at 3·25 kHz and on the styliform body at about 5·5 kHz. The styliform body region appears to be driven by coupling from the folded body resonance, but at its own resonance is relatively ineffective at driving the folded body which has become mass-limited.

When the tympanum vibrates, Müller's organ vibrates on the tympanum and shows complex rotatory motion and squeezing strains of its attachments

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to the tympanum. The strains differ in different parts of Müller's organ and change with frequency. Tuning curves of the strains differ from those of the tympanal vibration. The strains appear to be the basis of adequate stimulation of the receptors.

When a simple mass load is attached to the folded body its resonant frequency falls, but removal of the mass of Müller's organ does not cause an increase in the resonant frequency of the folded body, although the amplitude of its vibration increases markedly. Müller's organ thus acts as a damping or resistive load on the tympanum. The change in sharpness of tuning of the resonance of the folded body region indicates that about half the power required to sustain the vibration of the tympanum is dissipated in Müller's organ.

Müller's organ has different frequency-dependent drives acting on it, which are normal to each other. The resulting motion resembles a Lissajous' figure. Such figures can be measured along different axes to produce different tuning curves. There are three groups of receptor cells in the 'ganglion' region of Müller's organ oriented normal to, at 45° to and parallel to the tympanum. It is suggested that these are driven by different adequate stimulus spectra; this is borne out by independent work on the response spectra of single receptor cells.

INTRODUCTION

The ears of locusts are paired structures situated at the anterior end of the first abdominal tergite of the adult. Each ear has a thin cuticular tympanum bearing a series of sclerites to which are attached the sensory cells of Müller's organ. Müller's organ is connected by the auditory nerve to the metathoracic ganglion but is otherwise mechanically disconnected from the rest of the body. The inner face of the tympanum and Müller's organ are covered by the cuticle of one of a series of air sacs situated between the ears.

That locusts are able to discriminate the pitch of sounds was initially indicated by the work of Horridge (1960). The later work of Michelsen (1971*a*, *b*) described different response spectra from cells situated in different parts of Müller's organ and frequency-dependent patterns of tympanal vibration in a sound field. As a result of this work, it has been generally accepted that the locust ear shows a 'place' frequency discrimination analogous to that of the mammalian cochlea.

The physical basis of the 'place' discrimination theory of Michelsen is that the frequency-dependent modes of vibration of different parts of the tympanum interact to produce antinodes at or near the points of attachment of the sensory cells. Michelsen (1971*a*, *b*) correlated the frequencies and positions at which antinodes were observed in his holograms of tympana with the centre frequencies and anatomical location of the different sensory cells from which he recorded tuning curves.

The sensory cells of Müller's organ are connected anatomically to the tympanum at the sclerotized folded body, elevated process, styliform body and pyriform vesicle (Schwabe, 1906; Gray, 1960). The functional role of these sclerites appears analogous to that of the ossicular chain in the mammalian ear as transmitters of vibration of the tympanum to the sensory organ for neural transduction. Michelsen's model suggests that these sclerites might act as passive observers of the tympanal motion and transmit

the already frequency-dependent motion to the different groups of sensory cells. This view of the ear would be quite valid if the mass and compliance of the tympanum were substantially greater than those of Muller's organ. In such a case the tympanum would act as a 'constant current' source and determine the frequency response of the whole system. Further, because the source impedance would greatly exceed the load impedance, the proportion of the acoustic power that could be transduced would be small.

Vibration of Müller's organ has been observed when the ear is driven by sound (Michelsen, 1973) though this vibration was not described in detail nor was it correlated with the motion of the sclerites of the tympanum. Michelsen writes elsewhere (1971*b*): 'the excitation of the receptor cells is likely to be determined by the displacement of the dendrites *relative* to the rest of the cells. Therefore it is important to know whether the mass of cells in Müller's organ can be set in vibration by the movements of the tympanum.' Because of problems of spatial resolution, Michelsen (1971*b*) was unable to examine the relative motion of different parts of the ear in detail.

The anatomy of the tympanum, its sclerites and Müller's organ have been described by Schwabe (1906), Gray (1960) and Michelsen (1971 *a*, *b*). Their observations suggest that the masses of the sclerites and Müller's organ are similar to or even exceed those of the membranous parts of the tympanum. If this is the case, support is gained for Michelsen's (1973) suggestion that Müller's organ does not act as a passive observer of the tympanal motion; the mass and compliance of all the structures attached to the tympanum will affect the mechanical frequency response of the whole system.

The functional role of the ear is the transduction of sound into frequency-coded neural impulses. To achieve maximum sensitivity – and the locust ear appears to be of a similar order of sensitivity to mammalian ears (Michelsen, 1971*c*) – transduction of tympanal motion should be as efficient as possible, which would be met if the mechanical impedances of the system were appropriately matched.

The preceding discussion suggests bases for re-examination of the model of the locust ear proposed by Michelsen (1971 *a*, *b*). There is no doubt that the tympanum will play a role in determining the frequency response of the ear, but the anatomical evidence suggests that the mechanics of the other structures will also be significant. This can only be resolved by direct measurement of the distribution of mass and compliance within the system and of the motions of different parts of the system when driven by sound.

MATERIALS AND METHODS

(A) *Animals*

Mature adult *Schistocerca gregaria* from a laboratory culture were used for all the experimental work. Adult females were used for most experiments because their bodies are slightly larger than those of the males.

(B) *Anatomical studies*

For examination of the skeletal anatomy, ears were excised and left overnight in solutions of either 2–5% NaOH or papain before washing and manipulation. Scale drawings were made using a camera lucida. Scale photographs were taken using a Cambridge 150 SEM.

For mapping the tympanum, ears were fixed in Bouin, wax-embedded, cut as 10 μm serial sections and stained in Masson's Trichrome. Every tenth section was measured at 100 μm intervals across its width and the data so obtained were entered on an outline drawing of the shape of the tympanum using a calibrated microscope which allowed measurements with an uncertainty of about 0·2 μm. Contours of thickness were then fitted by interpolation between these spot measurements and confirmed by re-examination of the sections.

The mass of cuticle and tissue in different parts of the tympanum was then estimated by weighing tracings of the different parts of the contour map, to obtain the areas, and multiplying these by the average thickness to give the volume. A density of 1000 $kg.m^{-3}$ was taken for the epidermal cells and of 1300 $kg.m^{-3}$ for the cuticle of the tympanic membrane (Wainwright *et al.* 1976).

(C) *The ear preparation*

For most experiments the right ear of the animal was used. This was mounted as follows: the holder was the tip of a 1 ml disposable syringe, drilled out at the needle fitting and cut off obliquely to form a short tube of maximum length 5 mm and bore 3 mm. All but about 8 mm of the barrel of the syringe was also removed, so the overall length of the holder was about 13 mm. This holder was waxed securely to the side of the insect's abdomen and thorax around the ear in an orientation that placed the plane of the tympanum parallel to the axis of the tube. The ear was then dissected out from the insect and dusted with corundum granules of diameter between 2 and 10 μm using a single squirrel hair. It was possible to apply granules so that a conspicuous one was situated within about 25 μm of a desired part of the ear; its position was recorded on an outline drawing similar to Fig. 4. The total load applied thus was around 100 granules each of maximum mass around 0·1 ng, which is trivial in comparison with the 5–10 μg mass of the tympanum. The process of waxing and dissecting the ear took less than 10 min and the final two stages were normally completed in under 2 min.

For other measurements, holders with different tip angles were made so that the tympanum could also be mounted normal to the axis of the holder.

(D) *Compliance measurements*

A force balance was made from two 10 mm lengths of watch hairspring, glued at their inner ends 4 mm apart at the edge of a 15 mm piece of microscope slide glass. Their outer ends were glued to a 0·8 mm diameter tube rolled from 25 μm aluminium foil. A loop of 40 μm Nichrome wire was glued into one end of the aluminium tube to carry calibrating weights. A wire test probe of 120 μm diameter was waxed into the other end (see Fig. 1).

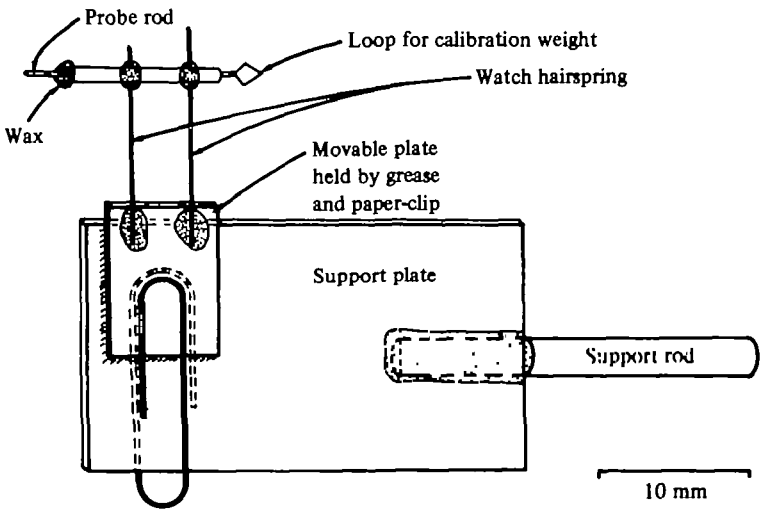

Fig. 1. Diagram of the force transducer used to measure compliance of parts of the ear. In use, the transducer was mounted on a Narashige micromanipulator so that it could be advanced by known distances along the axis of the probe rod. The transducer is assembled with Araldite, shown stippled in the diagram.

The balance was calibrated by hanging weights on it when the probe rod was vertical. The deflexion was measured for loads up to 500 μN and about 300 μm deflexion, which was reversible and beyond the normal operating force. The calibration was linear and stable over this range. The balance compliance was 1·73 $N.m^{-1}$.

For compliance measurements, the balance was clipped to a glass slide mounted on a Narashige micromanipulator with a micrometer head calibrated every 10 μm. The movement of this micrometer was measured with the calibrated microscope (E iii); errors around the measuring system did not exceed 5 μm in 500 μm.

For compliance measurements, the balance was arranged so that the probe wire could be advanced normal to a chosen point on the tympanum under examination. The tympanum, in turn, was arranged so that its plane was approximately parallel to the optical axis of the measuring microscope. The probe was advanced to within about 100 μm of the tympanum, the microscope was zeroed on a point on either the test probe or the ear, and the balance was then moved in steps of either 10 or 20 μm. This movement was measured down the microscope to the nearest 1 μm and recorded. The distance moved to contact with the tympanum was noted. The balance was then moved stepwise for a further 300 μm and the resultant movement of the reference point was recorded. The difference between the distance moved by the balance and that observed at the probe was used to calculate the force applied by the probe. The distance moved by a point on the ear can then be used to calculate the compliance at that point.

Readings could be taken at about 10 s intervals, so a complete set of measurements at a single point could be completed in under 5 min.

Compliance was also measured by applying air pressure to one side of the tympanum and measuring the deflexion normal to its plane. For these measurements, ears were

mounted as in section C above, particular attention being paid to sealing of the ear to the holder and of the adjacent spiracle. The mounted ear was fitted into a tube attached to the stage of the measuring microscope and sealed to a tube connected to a water manometer and a 5 l flask, the pressure in which was altered by one experimenter who blew or sucked air in or out of the flask.

Combined measurements were also made in which the tympanum was pressurized from one side and pushed by the force balance from the other side.

(E) *Measurements with closed field sound (see Fig. 2)*

(i) *Sound source*

It is rather difficult to produce high sound pressure levels over a broad frequency spectrum. We have used (and broken!) a variety of commercially available loudspeakers. The final one, which survived the experiments, consisted of a Whiteley Brothers (W.B. Stentorian) WB T10 horn tweeter. The horn was removed and replaced by a 35 mm long perspex block which allowed the loudspeaker to be fitted through the stage of the measuring microscope. The perspex block was bored to the 16 mm diameter of the hole in the loudspeaker and to a depth of 25 mm. The top 10 mm was bored to 6 mm diameter to take the holders of the standard ear preparation (see C above). The ear on the holder was pushed as far and as tightly as possible into the perspex block.

The loudspeaker was driven from the oscillator (see E ii) via a Hewlett-Packard 350D attenuator into a Quad 303 power amplifier. Performance was measured by fitting a Bruel and Kjaer $\frac{1}{8}$ in microphone, type 4138, on a type 2619 preamplifier into the top of the loudspeaker, sealing it in place with a short length of soft p.v.c. tubing. Moving the microphone up or down through 5 mm did not produce more than 0·5 dB change in its output, measured on a type 2607 measuring amplifier. The properties of the cavity were adjusted by inserting small tufts of cotton wool until the frequency response was reasonably flat over the range from 1·75 to 8 kHz. Over this range, 120 dB peak sound pressure (117 dB rms) was produced. Over the narrower band from 1·75 to 6 kHz, 125 dB peak (122 dB rms) could be produced, while by removing the cotton wool damping far higher sound pressures over more restricted bandwidths could be obtained (see results section C i). Distortion was measured by feeding the output of the B & K 4138 via the 2607 amplifier into a Radiometer wave analyser type FRA 2B. Below 1·5 kHz the waveform became ragged, but it became cleaner at higher frequencies. The total harmonic distortion at 122 dB rms sound pressure was 1·7% (−35 dB) at 2 kKz, dropping to below 0·3% (−50 dB) between 2·5 kHz and 6 kHz.

The source is a pipe of small diameter compared with the shortest sound wavelength. The wave propagated up it will thus approximate closely to a plane wave. We have made this assumption. The sound pressure levels we cite throughout are expressed in decibels (dB) above the accepted threshold of 10^{-12} W.m.$^{-2}$ or 2×10^{-5} N.m^{-2}. Although we calibrated the apparatus to an accuracy of $\pm \frac{1}{2}$ dB, there is some uncertainty about the sound pressure level within the ear holder. This is effectively a small blind tube set in a baffle; the diameter of this tube is only $\frac{1}{15} \lambda$ at the highest frequencies and so the effect on phase will be negligible. There will, however, be an

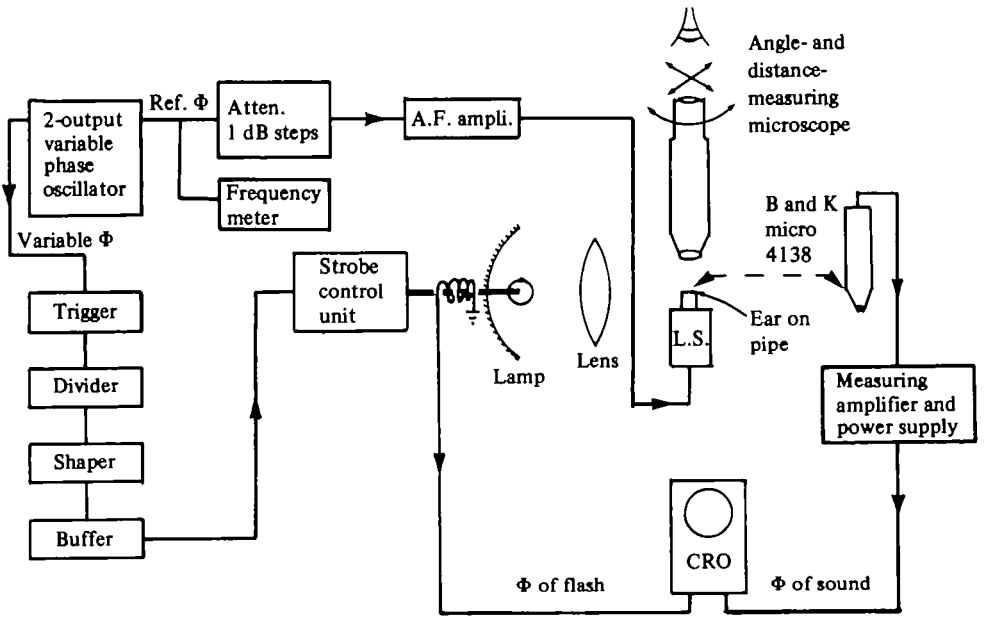

Fig. 2. Schematic diagram of the apparatus used to illuminate and deliver sound of known but variable phase difference to the outside of a prepared locust ear. The ear is mounted under a measuring microscope on a loudspeaker driven by one output of a 2-output oscillator, the other side of which drives a strobe lamp. The B. & K. microphone can be inserted in the loudspeaker instead of the ear for calibration of the pressure and phase of the sound.

effect on the intensity of the sound at the tympanum. The tube acts as an acoustic transformer, increasing the sound pressure over that measured in our calibration technique. From Olson (1957), it appears that the effect will not exceed 1–3 dB, even at the shortest wavelengths used.

(ii) *Phase-locked illumination* (see Fig. 2)

The oscillator was an Exact model 337 digital phase generator. One output was used to drive the loudspeaker and the other was arranged to drive a Dawe 1203 B Strobosun lamp. To this end, the output from the oscillator was fed into a digital dividing chain which consisted of the following Neurolog units: NL 200 spike trigger, NL 603 counter (which can be set to pulse after counting from between 1 and 99), NL 403 delay-width (to shape the pulse so that the strobe lamp would fire) and NL 510 pulse buffer. Using this chain, the lamp could be set to fire flashes at between 50 and 100.s^{-1} when the oscillator was running at between 1 and 15 kHz.

The frequency was set to ± 5 Hz using an Advance TC9A frequency meter. The phase of the flash with respect to that of the sound was observed by comparing the output from the calibrating microphone (section E i) with that from a 50-turn pick-up coil wound around the lead between the strobe power supply and the lamp unit. The phase of the flash was then adjusted at the variable-phase output of the oscillator until it occurred at the peak compression of the sound waveform; this phase angle was recorded and used as a reference in subsequent analysis of data. The settings of the

strobe-driving equipment remained stable with 5° phase angle over a two-month period, though the settings were checked repeatedly. The phase error of the microphone and amplifiers is less than 5° over the bandwidth of our experiments (makers' information).

The ear under examination was illuminated from the side by focusing the output of the strobe lamp though a pair of 200 mm diameter, 170 mm focal length glass Fresnel lenses. This gave more than sufficient light for visual examination and photography. The Fresnel lenses acted as heat filters; no heating effect could be detected.

(iii) *Measuring microscope*

A Watson Service II microscope was used throughout. The condenser was removed and the loudspeaker was bolted via an adaptor plate to the mechanical stage; this allowed the ear to be mounted and then centred.

Measurements were made with a Malies Instruments Curtain Measuring Eyepiece. For compliance measurements, a Reichert No. 3 Objective was used and the microscope was calibrated to 1 μm per unit at the eyepiece. For other measurements, a Baker $\frac{1}{2}$ in objective with a 3 mm working distance was calibrated to 0·75 μm per unit. The calibration was adjusted to give an overall accuracy of $\pm 0{\cdot}5\%$ or $\pm$ one eyepiece unit.

A protractor was attached to the microscope and a pointer to the eyepiece. Angles were measured to the nearest $\frac{1}{2}°$. The position of a point was defined by its range and bearing from the axis of rotation of the eyepiece. Initial setting of the microscope was made using a tungsten-wire pointer mounted on a micromanipulator, which was adjusted till the pointer tip was at the axis of rotation of the eyepiece.

The microscope and micromanipulators were mounted on a massive steel baseplate isolated from the bench by a bubble pad. Drifts during experiments rarely exceeded 1 μm. Vibration within the apparatus could not be detected. We were unable to detect any vibration of the cuticular rim of an ear driven at 122 dB rms sound pressure at 3·5 kHz, even though the peak-to-peak vibration of the tympanum exceeded 50 μm.

(iv) *Analysis and reconstruction of the observed movements of the tympanum and Müller's organ*

All the movements of single carborundum granules we observed were periodic, and varied from harmonic movements along a single axis to complex two-dimensional loci. All movements were reconstructed from measurements of the range and bearing of the granule in question from the centre of rotation of the microscope eyepiece. In the case of a linear movement along a single axis, the reconstructed movement could be closely approximated by a pure sine wave. This allowed the peak-to-peak amplitude to be specified to within $\pm$ 1 μm and the phase of the peak excursion to be defined to within 10°. In the case of movements which had more complex loci, tests on fixed points have shown that the method we have used allowed the position of any point in the locus to be defined to within a circle of diameter 3 μm.

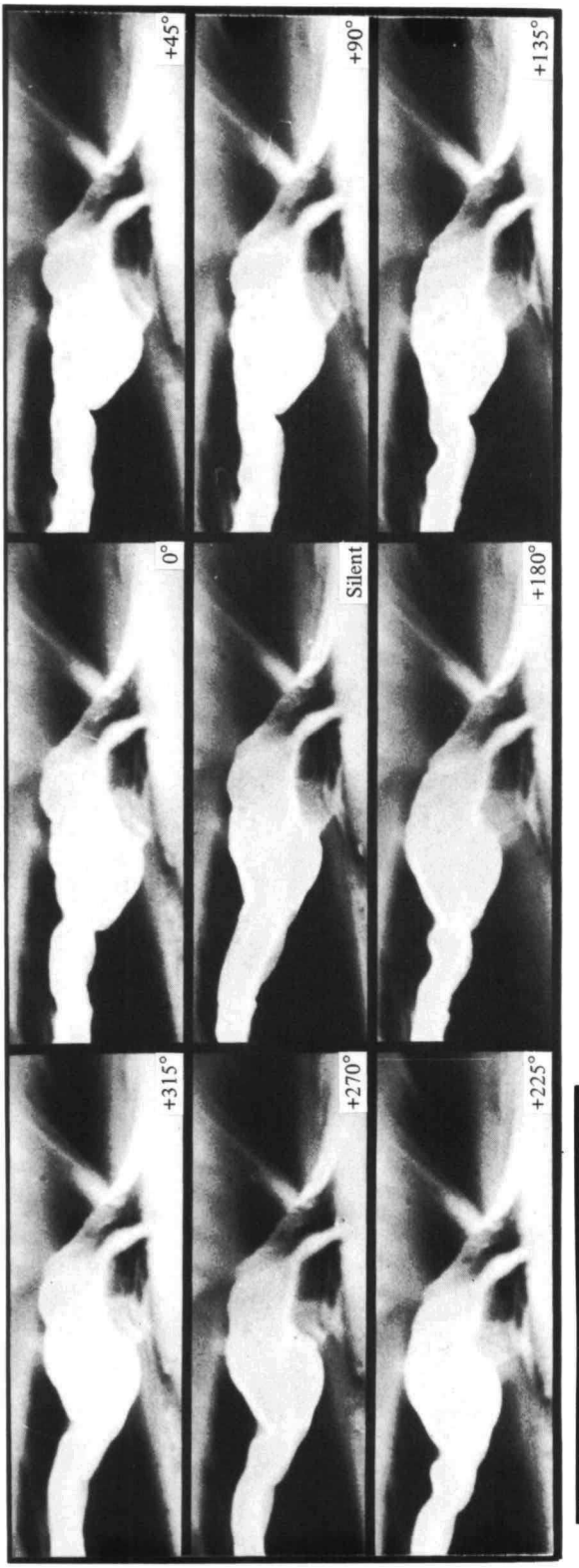

Fig. 3. Photographs of the movement of the tympanum and Müller's organ in a sound field of 122 dB rms pressure at 3·5 kHz. The pictures were taken by stroboscopic illumination phase-locked to the sound wave; the phase of the flashes with respect to the peak positive sound pressure (that causing inward and hence upward movement of the tympanum) is indicated on each picture. Each picture is made with an exposure of 8 s and is the sum of about 400 superimposed exposures. Anatomical details of the tympanum and Müller's organ are shown in Figs. 4 and 5.

(F) *Photography*

Owing to the compromise between resolution and depth of focus at high magnifications, photography was of little use for analytical purposes, though it serves to illustrate the types of movement we have observed (Fig. 3).

(G) *Mechanical loading and amputation*

Loads made of short lengths of 40 μm diameter Nichrome wire were attached to the tympanum using silicone grease. The mass of the load was obtained by weighing a 50 mm length of the wire and then cutting it in short lengths and selecting a piece of appropriate length (0·9 mm) and hence mass. This was then bent into a U shape, and glued in place with a minute smear of grease. After the experiment, the ear was inspected to check that the wire had not moved from the point of attachment; there was never any evidence that this occurred.

In other experiments we removed Müller's organ using forceps on the standard ear preparation. The cuticle of the air sac is broken during the operation. Operated ears dry out more rapidly than intact ears, so the range of observations that can be made is more limited.

RESULTS

(A) *Anatomy of the ear*

(i) *Gross anatomy of the tympanum, its sclerites and Müller's organ*

Although the anatomy of the tympanum has already been described by others (e.g. Schwabe, 1906; Gray, 1960; Michelsen, 1971*a*, *b*), it is appropriate to give a brief general description and also to report new anatomical studies which were necessary for interpretation of the observations made in this study.

Masson's Trichrome stains different types of cuticle characteristically (see e.g. Neville, 1975). Endocuticle, which is normally compliant, stains blue. Mesocuticle, which has smaller intermolecular spaces due to impregnation with proteins or lipids and so is presumably stiffer, stains red. Tanned or sclerotized exocuticle, which is very stiff and brittle, does not stain. The differential staining behaviour gives an indication of the cuticular properties. The epicuticle does not stain but is usually far thinner than the other cuticular components; the mechanical properties are little known.

The tympanum is surrounded by a plane oval rim of thickened and sclerotized cuticle. It is about 2·5 mm high, dorso-ventrally, and about 1·5 mm wide. It is divided by a row of sclerites running dorso-ventrally about 0·5 mm in from the anterior edge of the tympanum. The thick anterior part of the tympanum appears opaque. The far larger posterior inner part of the tympanum is almost transparent and is thin.

Previous workers have not illustrated the sclerites in any detail (see Fig. 4). The most dorsal, the styliform body, arises as a sclerotized region of the tympanum. Dorsally, it becomes thinner and wider and merges into the cuticle of the tympanum. Ventrally, it narrows and joins, by a neck of meso- and endocuticle, to the elevated process. Near this neck, a bar or process projects out from the foot over the top of the elevated process; this process is sclerotized near the tympanum but becomes meso-

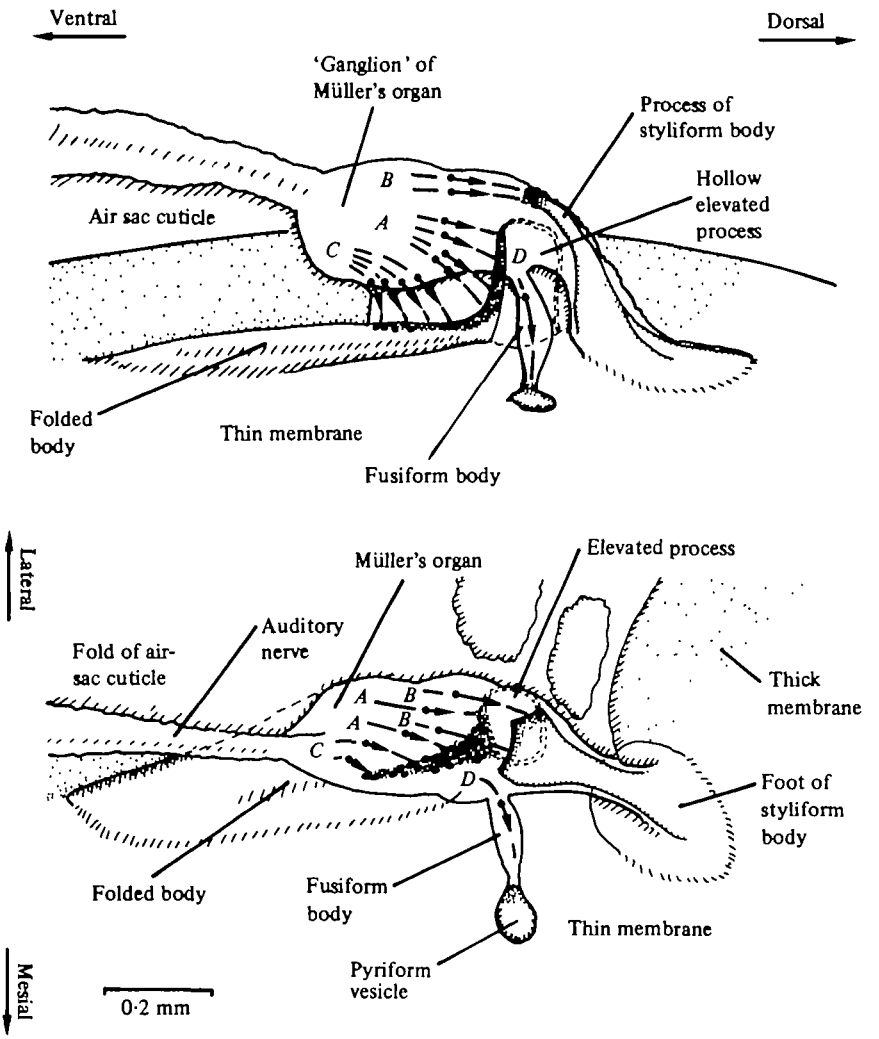

Fig. 4. Drawings of the tympanal sclerites and Müller's organ to show the trajectories of the four groups of sense cells. The points of attachment to the sclerites are shown by small circles and stippling, the approximate length of the scolopale cells is indicated by the length of the arrows and the position of the groups of cell bodies is given by the letters *A–D*. The drawing is built up from camera lucida drawings of whole ears, serial sections and the published accounts by Schwabe (1906) and Gray (1960). Upper: viewed from about 10° above the plane of the tympanum. Lower: viewed normal to the tympanum. The relation of these drawings to the whole tympanum can be seen from Fig. 5 or 6.

cuticle about half-way along its length and has an arciform section. The elevated process is tubular and has a tuberculate epicuticle. The bulk of the wall thickness is made up of mesocuticle lined by endocuticle, which feels quite soft when manipulated by forceps. On the lateral side, the elevated process merges with the soft endocuticle of the 'thick membrane' and, on the mesial side, with the thin mesocuticle that surrounds the pyriform vesicle. Ventrally, the cuticle of the elevated process merges with the sclerotized cuticle of the folded body. The folded body is a rigid sclerotized

plate, which becomes thinner at its ventral end and is folded along its lateral edge where it joins the 'thick membrane'. The smallest sclerite, the pyriform vesicle, is highly sclerotized and is situated on the 'thin membrane' about 0·3 mm from both the foot of the styliform body and the elevated process. The form and dimensions of these sclerites are shown in Fig. 4.

Müller's organ (Fig. 4) is attached to the sclerites and contains about 80 sensory units, each with a cell body in the 'end-organ' (Schwabe's terminology, 1906) or 'ganglion' (Gray's terminology, 1960) and a sensory cilium surrounded by a scolopale cell attached via a specialized cell to the epidermis. The sensory attachments on to the sclerites occur in four groups (Gray, 1960). The trajectory of these and the approximate position of the scolopale cells are shown in Fig. 4.

The whole ear is covered by a thin-walled air sac. This does not appear to move relative to the ear, to which it is closely applied. The auditory nerve passes from the cuticular rim of the ear within a fold of the air-sac cuticle, which in many specimens enfolds the thick membrane of the tympanum and Müller's organ.

The fusiform body is roughly ellipsoidal, about 100 μm long and 50 μm in diameter. The region containing the cell bodies is about 400 μm long and 200 μm in diameter, but these dimensions vary considerably from individual to individual. The estimated mass of the fusiform body is 1 μg and of the cell body region between 8·5 and 12·5 μg. In addition, there is a length of nerve and an extensive fold of tissue connected between the cell bodies and the cuticular rim. As these do not appear to be important in the mechanics (see Results D i), no attempt has been made to estimate their mass.

(ii) *Variation in thickness of the tympanic membrane and the consequent distribution of mass*

The tympanum shows systematic variation in thickness across its surface. The epidermis shows a similar variation in thickness. Fig. 5 shows a contour map built up as described in Methods B.

There are two thickened regions on the thin membrane, one around the folded body and the other around the styliform body. These are separated by an arc of thin cuticle from the mesial rim of the ear and from each other by an oval area of thin cuticle that surrounds the pyriform vesicle. The thick membrane extends from the lateral edge of the ear to the folded body, elevated process and about half-way up the dorso-lateral edge of the styliform body.

The whole tympanum is covered by an epicuticle, the thickness of which does not appear to exceed 0·2 μm. In the thin membrane there is also a layer of mesocuticle. Approximately half the thickness of the thick membrane is mesocuticle and half endocuticle; the extent of the endocuticle of the thick membrane and elevated process is shown stippled on Fig. 5. The epicuticle of the thick membrane and air sac has stellate folds, which are characteristic of extensible cuticle (see Wigglesworth, 1933).

The whole of the tympanum is lined by the cuticle of an air sac. This appears to consist solely of epicuticle which is not thicker than 0·2 μm.

Assuming a density of 1300 $kg.m^{-3}$ for the cuticle (Wainwright *et al.* 1976) and 1000 $kg.m^{-3}$ for the epidermis, we have estimated the distribution of mass over the surface of the tympanum. The areas into which the tympanum has been divided for

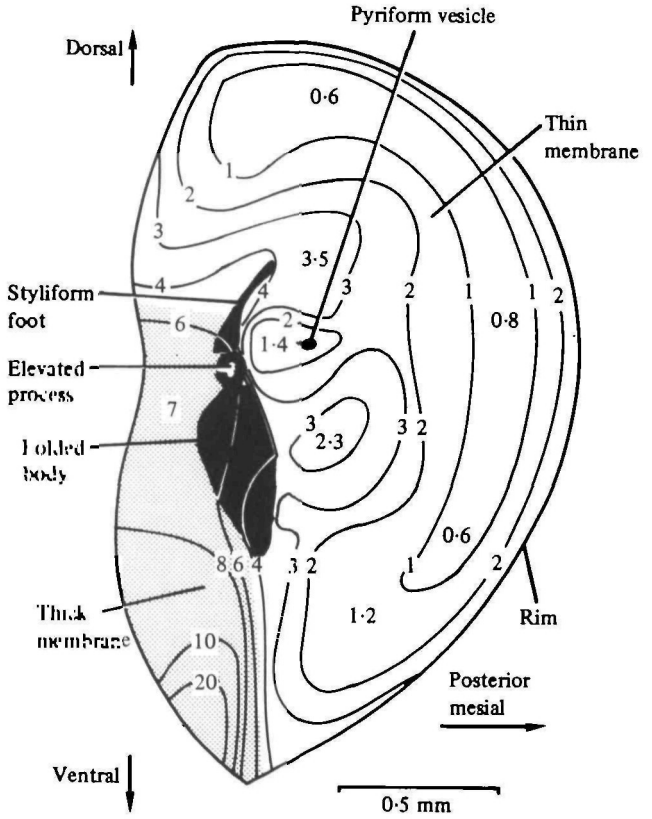

Fig. 5. Map of thickness of the external cuticle of the tympanum built up from serial sections. Contour lines are drawn with occasional spot thicknesses. Sclerotized exocuticle is shown black, the 'thick membrane' with its stellate-folded epicuticle mesocuticle and endocuticle is stippled and the 'thin membrane' with epicuticle and mesocuticle is unshaded. In this map the thickness of the epidermal layers and air sac epicuticle are not shown.

the purposes of this estimate are shown in Fig. 6. Since the external dimensions of the original tympanum and its reconstruction correspond closely, we believe that little shrinkage occurred.

Table 1 shows our estimates of the weights of different parts of the ear. Michelsen (1971*b*) gives the weight of the entire tympanum, apparently including Müller's organ, as 30 μg (weighed) and 40–45 μg (estimated) and of the thin membrane as 7·7 μg (weighed) and 10·8 μg (estimated). Our estimates of 10·7 μg for the thick membrane, 10·1 μg for the thin membrane and 10·5 μg for Müller's organ give a total and partial weights which agree well with Michelsen's values.

(B) *Static compliance of different parts of the ear*

(i) *By forces applied to discrete points on the tympanum*

We made compliance measurements on different parts of the tympanum using a total of 15 animals. Typical raw data from these measurements gave plots of the type shown in Fig. 7, which shows the consistent slopes of the stress–strain data and

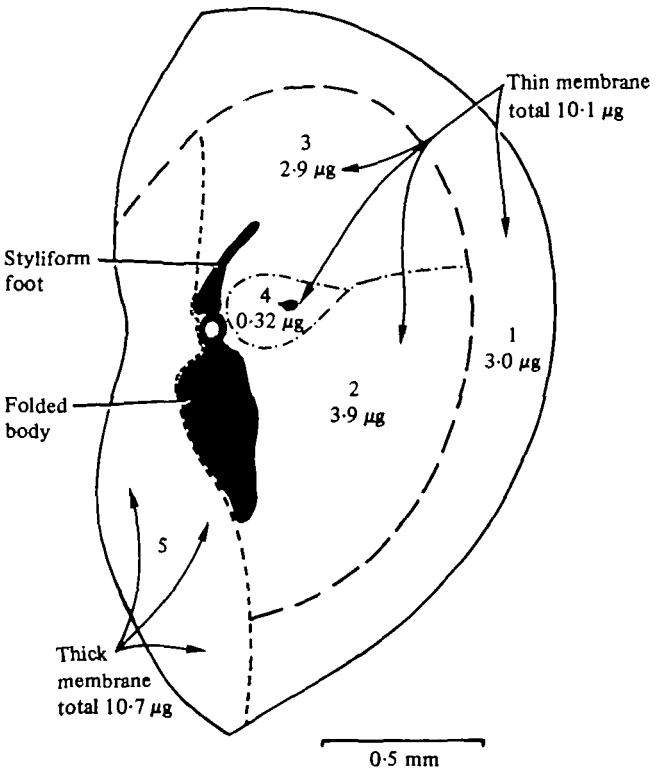

Fig. 6. Map of the distribution of the mass of the tympanum. This is calculated from the thickness map of Fig. 5 with the addition of the estimated mass of the epidermal layers and the air sac epicuticle, as indicated in Table 1. The areas labelled 1–5 have been used to calculate resonant frequencies as shown in Table 4 (Discussion, section B).

Table 1. *Estimated area and mass of different regions of the tympanum*

Region (see Fig. 6)	Area ($m^2 \times 10^{-6}$)	Mass of cuticle (μg)	Mass of cells (μg)	Total mass (μg)
Thin membrane				
Arc near rim, 1	0·75	1·5	1·5	3·0
Area around folded body, 2	0·76	2·4	1·5	3·9
Area around styliform body, 3	0·60	1·7	1·2	2·9
Area around pyriform vesicle, 4	0·07	0·18	0·14	0·32
Total for thin membrane, 1+2+3+4	2·2	5·8	4·3	10·1
Thick membrane, 5	0·66	7·4	3·3	10·7
Total for tympanum	2·86	13·2	7·6	20·8
Müller's organ (Results, A i)	—	—	—	8·5–12·5

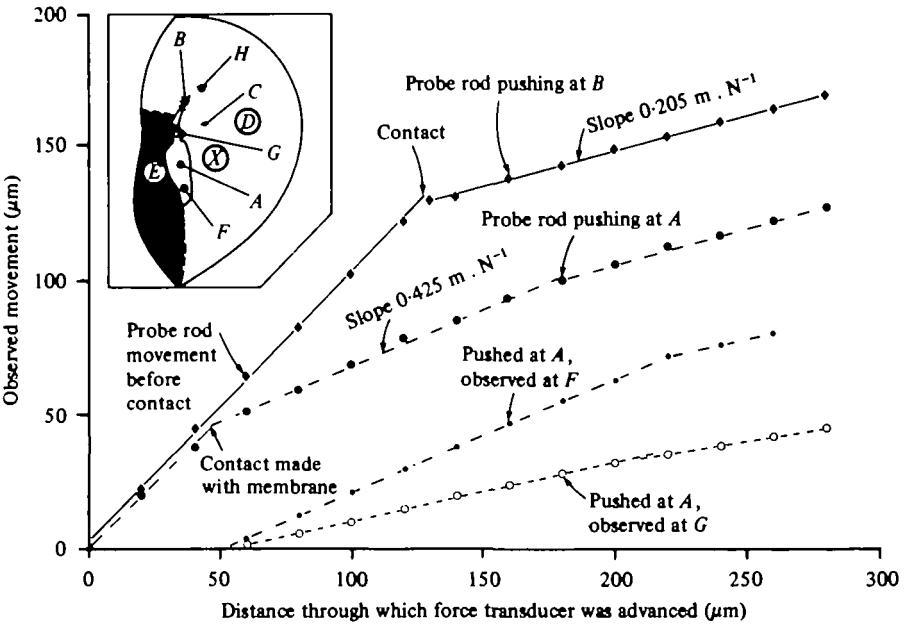

Fig. 7. Raw data of compliance measurements made at different points on a locust tympanum using the transducer shown in Fig. 1. The points examined are indicated on the inset, the method of measurement is explained in Methods, section D and the results are described and discussed in Results, section B i. The inset also shows the location of other points described in section B i.

differences in slope for different parts of the tympanum. Because of differences between the techniques, these raw data appear different from those of Michelsen (1971*b*), although we confirm that for initial strains of about 50–80 μm the compliance at any one point is constant and then more or less abruptly changes to a lower (stiffer) value. Between tympana there is considerable variation which we are unable to explain but which, because of the good agreement between measurements made within 5 min of initial dissection, 1 h and 12 h later, does not seem to be caused by drying of the preparation. Nor is the variation due to plasticity, since readings made as the force was increased corresponded closely with those made as the force was then decreased.

Because of the variability from animal to animal, we made measurements at several different points on each of several tympana; this gave the relative compliance of these points. For one tympanum for which such a set of readings was made, we show both the points at which measurements were made (Fig. 7, inset), some of the raw data, and the compliance values obtained (Table 2). The range of values obtained in other experiments is also indicated in Table 2.

The thick membrane is always more compliant than any part of the thin membrane. Its character, too, is different. Force applied at one point causes only local deformation, rather than movement of an area of the surrounding tympanum. In particular, deformations 0·2 mm from the folded body causes almost no movement of the folded body. It appears to act as a membrane.

The compliance of the styliform body is always less than that of the folded body, typically by a factor of $\frac{1}{2}$ to $\frac{3}{4}$.

The effect of local application of force on movement at other regions of the tympanum has also been examined. Force applied at the pyriform vesicle (point *C*, Fig. 7) causes little movement of either the folded body (*A*) or the styliform body (*B*). By contrast, when the centre of the folded body is moved (point *A*), a considerable area (*AXCD*) of the surrounding tympanum, including the pyriform vesicle (*C*), also moves, but the amount of movement falls with increasing distance from the folded body. Force applied half-way along the folded body (point *A*, Fig. 7) causes similar movement at the point of application and at the ventral end of the folded body (point *F*), but only about half as much movement of the elevated process (*G*) (see Fig. 8) and a very small movement of the styliform body (*B*). Similarly, force applied in the middle of the foot of the styliform body (point *B*, Fig. 7) causes a similar movement of the tympanum dorsal to it (*H*) but a far smaller movement of the elevated process (*G*) and folded body (*A*). The thick membrane (point *E*) is highly deformable, and application of force causes quite large strains to occur in it with little movement of the folded body (*A* and *F*).

The thickened areas of cuticle around the styliform body and elevated process thus appear to be able to act as independent plates, decoupled from each other by the elevated process. The thick membrane appears to act as a compliant outer-edge support for the thin membrane.

The compliance of Müller's organ along an axis normal to the plane of the tympanum about 150 μm ventral to the elevated process (above point *A*, Fig. 7) is 10–20 times that of the underlying tympanum (Table 2).

(ii) *By air pressure applied to one side of the tympanum*

A uniform force was applied to the tympanum by setting up pressure gradients of up to 200 mm H_2O (Methods D). Measurements were made at intervals of 10 mm pressure for points on the folded body, styliform plate, pyriform vesicle and middle of the thin membrane. Typical results are shown in Fig. 8, where it will be noted that the tympanum behaves approximately symmetrically. The compliance can be obtained from the instantaneous slope of the lines and two aspects are important. First, the compliance decreases with increasing strain, as would be expected of a tensed membrane. Secondly, the compliance is only about one-tenth that measured for similar parts of the tympanum when loaded locally.

When the tympanum is loaded all over, it bulges and its tension increases steadily with increasing load, decreasing its compliance with increasing pressure. When small areas are loaded, the strain is localized and determined by the local load and stiffness; in the present case, the areas around the styliform body and folded body behave as if they were plates suspended by springs with linear stress–strain relations and where, because the stress is more localized, the resultant strain may be far greater.

This point was illustrated in another series of measurements in which the compliance of a point on the tympanum was measured first by applying internal pressure, then by use of the force transducer and finally using the force transducer at the same point after the application of an internal pressure to the membrane.

Table 2. *Compliance of different parts of the locust tympanum. Measurements were made at points shown in Fig. 7*

Point on tympanum (refer to Fig. 7)	Typical compliance ($m.N^{-1}$)	Range observed ($m.N^{-1}$)
Folded body, *A*	0·38	0·27–0·46
Styliform foot, *B*	0·21	0·18–0·35
Pyriform vesicle, *C*	0·22	0·21–0·44
Thin membrane, *D*	0·22	0·17–0·25
Thick membrane, *E*	0·7	0·5–1·4
Müller's organ (see text)	7·5	4–9

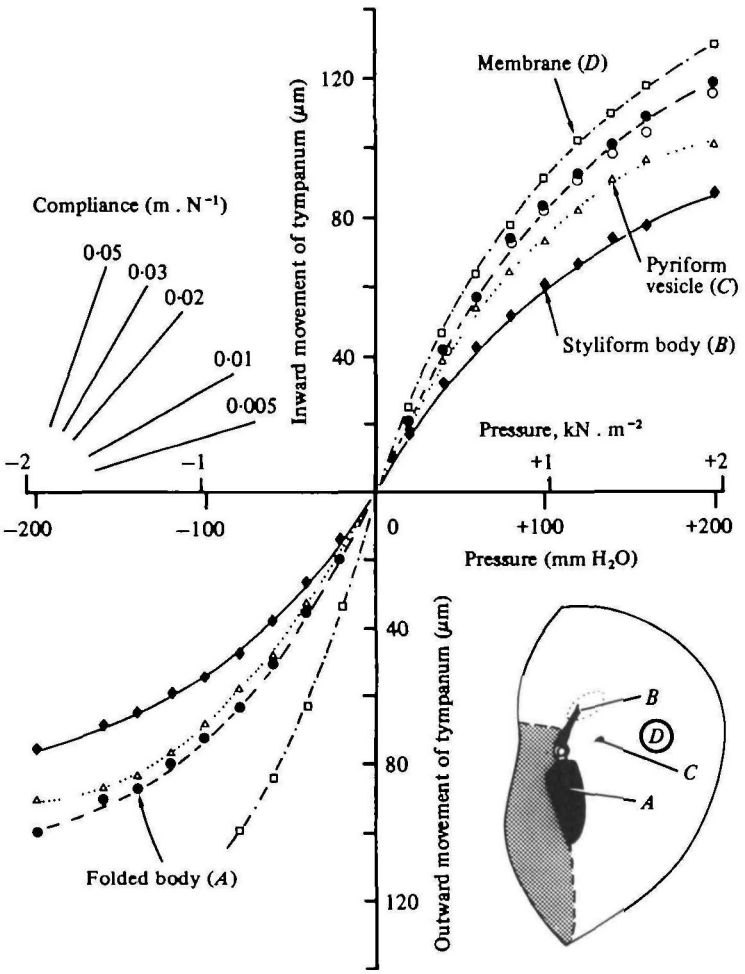

Fig. 8. Graph of the movement of different points on the locust tympanum when the whole ear is subjected to a static air-pressure gradient across the tympanum. Measurements were made in the order *ABCDA* with the open circles showing a repetition of the measurements shown by the closed circles but 30 min later. The pressure scale shows pressure in mm H_2O and in $kN.m^{-2}$. The straight lines in the upper left-hand quadrant show the calculated movement *vs.* pressure for membranes of the area of the tympanum and constant compliance. The diagram in the lower right-hand quadrant shows the location of points *A*, *B*, *C* and *D*.

Table 3. *Compliance measurements at a point on the tympanum mesial to the styliform foot*

Pressure gradient across tympanum (mm H_2O)	Compliance due to pressure gradient ($m.N^{-1}$)	Local compliance by force transducer ($m.N^{-1}$)
0	0·030	0·26
10	0·012	0·12
20	0·0068	0·072

The results are shown in Table 3. It will be noted that the compliance of the tympanum decreases with increasing pressure and hence increasing membrane tension, but that the values of the compliance obtained with the two methods differ throughout by an order of magnitude.

This experiment suggests that an increase in pressure across the tympanum increases the stiffness of the 'springs' supporting the thickened regions of the tympanum; the mechanical properties of the tympanum will be affected by ventilatory pressure.

C. *Vibrations of the tympanum*

As indicated in Methods E i, our measurements have been confined to the band between 2 and 6 or 7 kHz. The effects of different driving conditions on the intact ear are described here.

(i) *Constant frequency and varying sound pressure levels*

In a series of experiments, ears were driven at sound pressure levels between 102 and 132 dB rms. A calculated linear regression of log amplitude of vibration at the folded body *v.* log sound pressure level had a slope of 0·998 and regression coefficient of 0·996 for nine measurements at 3 dB intervals at 3·5 kHz. We were unable to detect any change in the phase between peak excursion of the tympanum and peak sound pressure over this range of sound pressures. Similar results were obtained at 2 and 4·5 kHz.

Since the motion is determined by the reactive mass and compliance and the resistive viscosity, the constancy of the input/output phase relations suggests that the reactive components remain constant, and the constancy of the input/output amplitude relations suggests that the resistive term remains constant. It thus seems reasonable to assume that the system does not show major amplitude-dependent nonlinearities and that the behaviour at 122 dB is similar to that at lower sound pressures.

(ii) *Varying frequency and constant sound pressure level*

The vibration of three different points on the same tympanum of an intact ear has been plotted over the full frequency range of the apparatus. The results, expressed in dB with respect to the maximum observed amplitude at 3 kHz are shown in Fig. 9A. For the point on the folded body there is a single peak around 3·0 kHz and a plateau in the decay of the vibration around 5 kHz. For the point on the styliform foot there are two peaks of similar amplitude, at 3 kHz and 5·5 kHz, separated by a con-

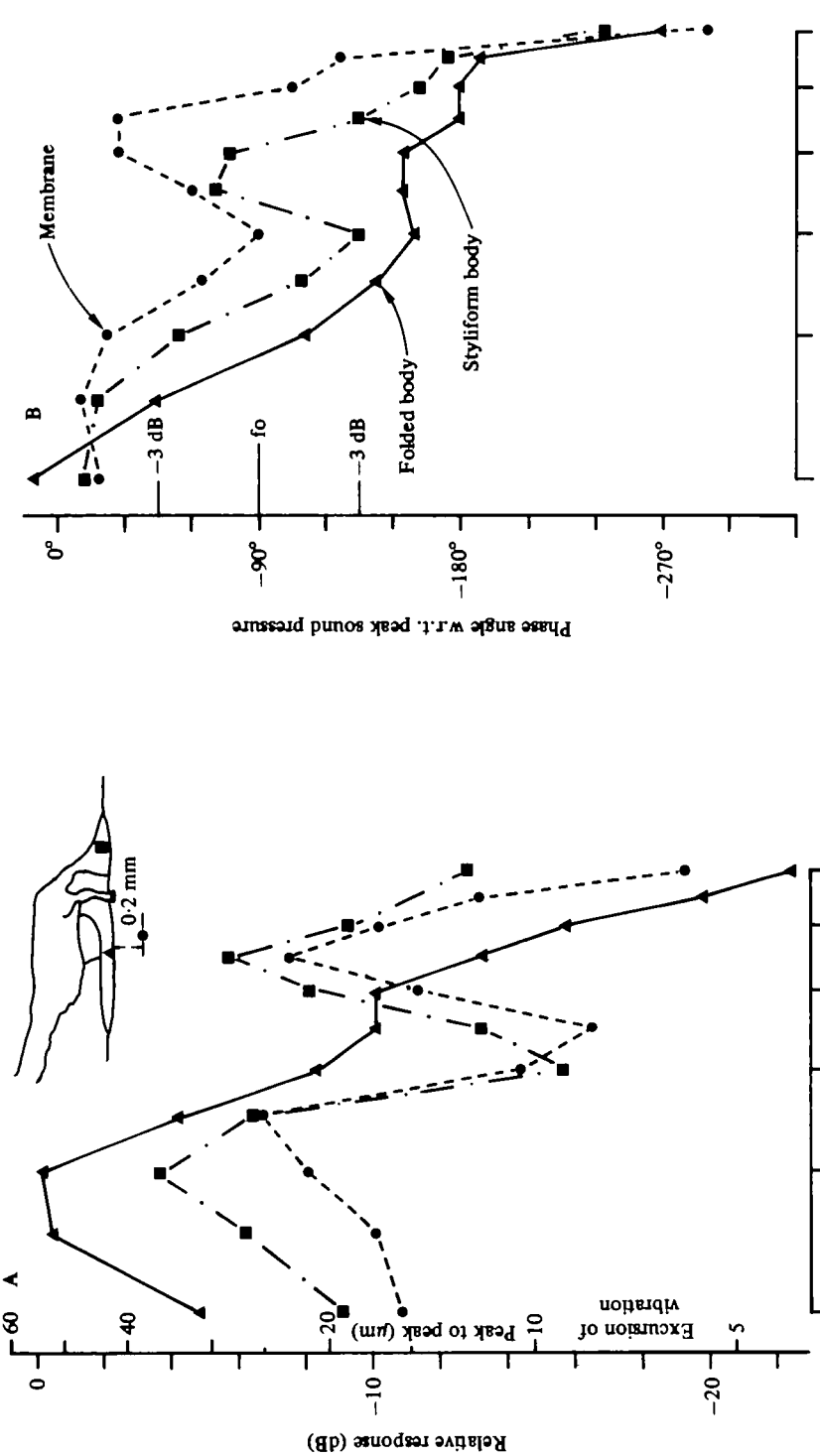

Fig. 9 (A). Graph of relative and absolute vibration of three points on the same tympanum for frequencies between 2 and 7 kHz and a constant sound pressure of 122 dB rms. The points measured are shown on the inset and correspond to the following points on Fig. 7: ▲ is point *A*; □ is point *B*; ● is point *X*. Relative amplitudes of vibration are referred to the peak amplitude observed at 3·0 kHz on the folded body. (▲) (B) Graph of phase of the peak inward movement with respect to the phase of the peak of compression of the driving sound wave for the vibration of the three points shown on Fig. 9A *vs.* the driving frequency at a constant sound pressure of 122 dB rms.

siderable trough between 3·5 and5 kHz. A point 0·2 mm out on to the thin membrane shows a similar behaviour to that of the styliform foot.

When the phase of these vibrations is related to that of the driving force of the sound wave, it can be seen (Fig. 9B) that there is a smooth swing from 0° through to 150° lag for the point on the folded body, followed by a further swing at about 6·5 kHz. The behaviour of the styliform plate is more complex; there is a lagging swing from about −15° to −130° between 2·5 and 4 kHz, followed by a forward swing to about −70° between 4 and 4·5 kHz, which is the region of the trough between the two resonances. There is then a lagging swing through to −160° on passing through the second amplitude peak at 5·5 kHz. The point on the thin membrane shows less phase lag than the two points on the sclerites up to 5·5 kHz, but then the phase swings rapidly through over 180°, suggesting that this part of the tympanum is passing through a true resonance, as the amplitude data (Fig. 9A) confirms.

Above 6·5 kHz, the phase at all three points swings through a further 90°, which may be due to the appearance of a rather sudden higher-order resonance. We have not been able to examine this further.

The vibrations we describe here are compatible with both Michelsen's (1971*b*) holograms and his capacitance-probe measurements.

The behaviour of the folded body region appears to be that of a mass-limited system (see Appendix). It is not clear, from this experiment alone, what type of loading will be presented by Müller's organ, but due to the substantial mass of Müller's organ it cannot be ignored. This point is examined further in section D iii.

(iii) *The force-amplitude relations of the vibration*

A sound pressure of 122 dB rms gives peak pressures of ± 36 N.m^{-2}. At 2 kHz, we have observed a vibration of the folded body of 30 μm peak-to-peak or ± 15 μm. Assuming that the sound pressure acts over an area of 0·76 mm^2 – the area of the plate-like region around the folded body (Table 1, area 2 on Fig. 6) – the calculated compliance is 0·55 m.N^{-1} compared with a measured value of 0·38 m.N^{-1} (Table 2). The motion, which well below resonance will be dominated by the stiffness, is compatible with the earlier observation that the region around the folded body acts as if it were a plate in an elastic surround (Results B ii). The maximum compliance of the tympanum measured by the application of a force over its whole area of 2·9 mm^2 (Results B iii) is about 0·05 m.N^{-1} (Fig. 8). A sound pressure of 122 dB rms acting on one side of a membrane of the observed compliance of the whole tympanum would produce a vibration of only 5 μm amplitude, which is far less than has been observed.

The observed amplitude of vibration is compatible with the suggestion, from the anatomy (Results A ii), the compliance measurements (Results B ii) and the amplitude and phase *vs.* frequency plots (Results C ii), that the area around the folded body is able to behave as a relatively independent unit.

(D) *The mechanics of Müller's organ*

Müller's organ is attached *via* the *c* cells to the folded body, *via* the *b* cells to the tip of the styliform body, and *via* the *a* cells to the elevated process (Fig. 7). There is also a thin connexion *via* the *d* cells to the pyriform vesicle. These connexions are extremely compliant (Results B i, Table 2).

(i) *The loci of different parts of the ear at a single frequency*

When Müller's organ is observed under slowly phase-slipping stroboscopic illumination, it can be seen to be undergoing considerable rocking and squeezing movements (Fig. 3). Internal strains, particularly in the hyaline region of the scolopidium level of the various groups of receptors, occur (Results A i). These movements do not, however, extend far up the auditory nerve. As these movements cause strains which could stimulate the sensory cells, we have examined them in more detail, but only in two of the three dimensions in which relative movements of tympanum and Müller's organ can occur.

Measurements have been made in a plane, passing through Müller's organ and the auditory nerve, normal to the tympanum (as seen in Fig. 4A) at four points on the surface of the organ and, with the same preparation, at four points on the tympanum at the single frequency of 3·5 kHz and a sound pressure of 122 dB rms. The loci of these eight points are shown on Fig. 10. It will be seen that the vibration of the points on the tympanum is approximately normal to its plane and the phase lags by between 60° and 120° on the peak sound pressure. The motion of Müller's organ is far more complex. Between point *A*, on the folded body, and point *B*, the direction of the *c* cells, there is some 90° phase shift, though the absolute amplitude is similar; thus there is a periodic strain of $\pm 40\,\mu$m here. Between point *B* and point *F* at the top of the elevated process there is both a relative movement normal to the plane of the membrane of $\pm 40\,\mu$m and a movement of $\pm 15\,\mu$m parallel to the membrane. Between the top of the elevated process, *F*, and point *G* on Müller's organ, the movement normal to the tympanum is rather smaller, but the movement parallel to the tympanum is far greater and approximately antiphase to that at point *B*, so there is a rocking movement between these two points. This can be seen also on Fig. 3.

At this frequency the *a*, *b* and *c* cells may all be strained. It is not possible, from these measurements alone, to say more than this

(ii) *Relative movements at different frequencies*

Since it is clear that the relative movements of the membrane and Müller's organ can provide a basis for stimulation of the sensory cells, a study of the frequency relations of these movements was made.

One series of experiments examined the loci of a point at the tip of the styliform body and, on the same ear, of a point on the surface of Müller's organ. The results are displayed in Fig. 11.

Fig. 10. Two-dimensional plots of the loci of vibration of eight points on the tympanum and Müller's organ made with a single ear at a constant 122 dB rms and at 3·5 kHz. The position of the points is shown on the diagram at the centre. The same scale is used for all plots with the individual origins of up–down and side–to-side axes showing the rest or no-sound position of the individual points. For each plot, measurements were made at 45° phase intervals, shown by solid triangles, the phase of the peak compression of the sound wave is shown by an open triangle and the direction of rotation is shown by an arrow placed about 25° before the peak sound compression. Where movement is in phase with the driving sound wave, the open triangle is expected at the highest point on the plot; the movement of point *A* lags by 90° and of point *B* by 180° on the driving sound pressure. The letters defining the points apply only to this figure.

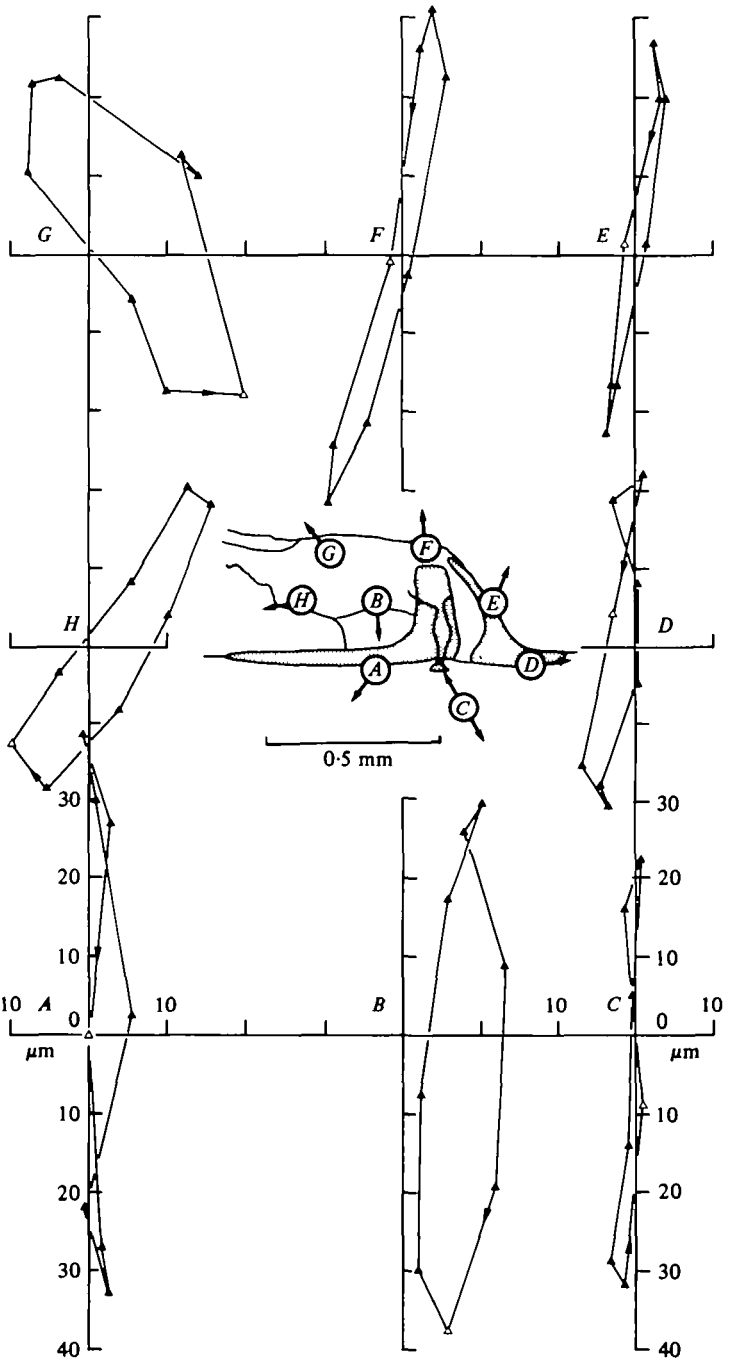

Fig. 10. For legend see opposite.

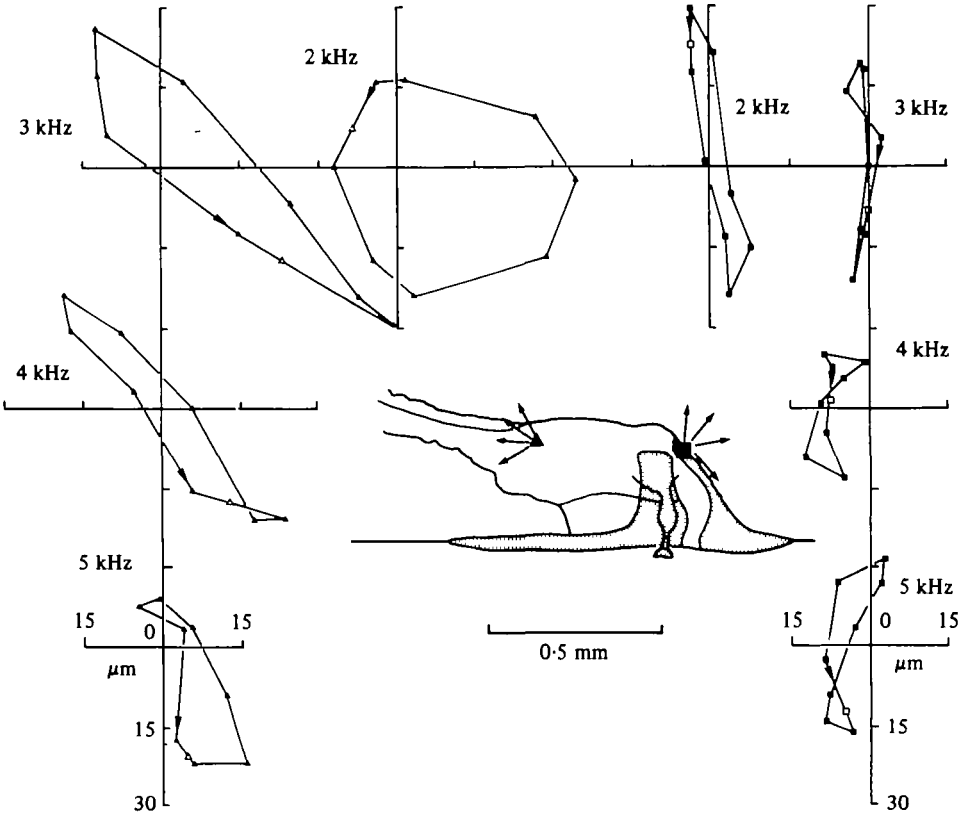

Fig. 11. Plots of the loci of the vibration of a point on the process of the styliform body (■) and a point on Müller's organ (▲) for a constant sound pressure of 122 dB rms and frequencies of 2, 3, 4 and 5 kHz. Open symbols indicate the phase of peak compression of the driving sound wave, and the cross-over point on the axes of each plot shows the rest or no-sound position for each plot. See also the legend for Fig. 10.

Increasing the frequency from 2 to 5 kHz causes significant change in the amplitude, phase and locus of the motion of both the point on Müller's organ and that on the styliform body. The locus of the point on Müller's organ is more or less circular at 2 kHz, with its centroid displaced some 15 μm towards the styliform process. As the frequency rises the locus becomes elongated and then shrinks, and its centroid moves towards the folded body. The locus of the styliform process is more or less linear and normal to the tympanum at low frequencies but at higher frequencies becomes a figure-of-eight, and its centroid moves towards the elevated body.

The relative phase of the two motions also shifts considerably over the frequency range.

Müller's organ shows a periodic motion and sustained displacement, both of which vary with frequency and both of which differ from the periodic motion and sustained displacement of the styliform body. The motion of the folded body differs from that of the styliform body (Fig. 9) and the two motions are applied to Müller's organ approximately normal to each other. When two such drives differing in phase and normal to each other are resolved vectorially, loci of the type seen here or Lissajous' figures are produced. In the present case, it would be hard to give a rigorous explana-

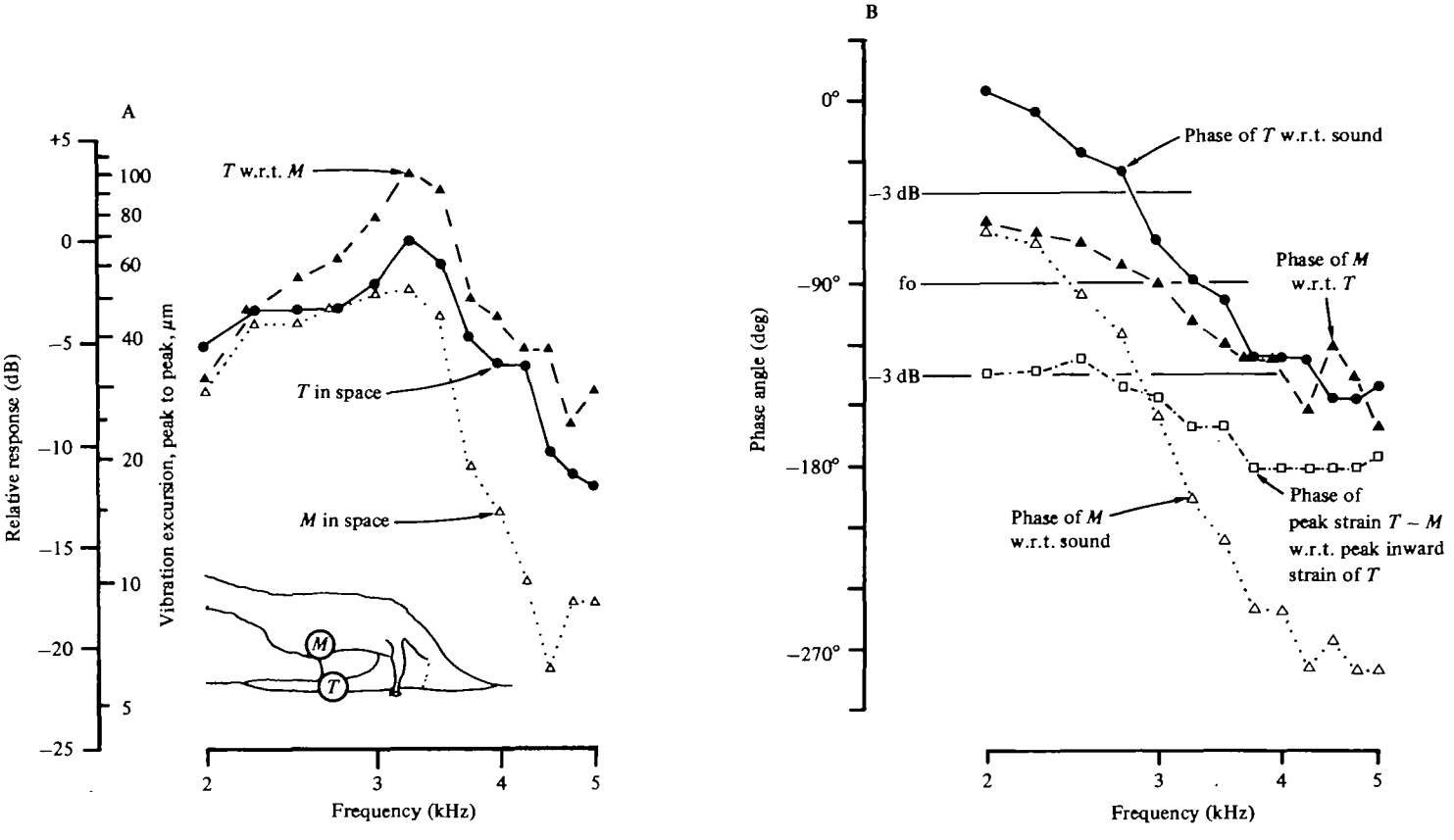

Fig. 12. Graphs of the vibration normal to the tympanum of a point on the folded body (*T*) and on Müller's organ (*M*) for a constant 122 dB rms between 2 and 5 kHz, made on a single ear.

(A) Plot of absolute excursion peak-to-peak in μm and relative amplitude in dB with respect to peak amplitude at point *T*. For the vibration of point *T* or of point *M*, an arbitrary fixed datum was chosen; the vibration of *T* with respect to *M* was plotted vectorially from the vibrations in space of *T* and *M*.

(B) Plots of the phase of vibration of the points with respect either to the phase of the sound or each other. The convention used to define phase is given in the Appendix. For a fuller description and discussion, see Results D ii.

tion of the loci of points in the middle of Müller's organ, because of phase differences not only between the motions of the sclerites but also within Müller's organ, which has mass and compliance both as a whole and between different regions, all of which will introduce frequency-dependent effects.

Because the strain appears to be relatively simple and is confined to a small region between Müller's organ and the sclerite, the easiest strain to study is that in the *c* cell region normal to the folded body. The vibration of points on Muller's organ and the folded body is more or less in a line normal to the plane of the tympanum. Fig. 12. shows the results of one such experiment. The vibration of the tympanum shows a peak at 3·25 kHz and a bandwidth at −3 dB of 0·75–1 kHz; though the amplitude of vibration peaks at a slightly higher frequency in this experiment, the curve is similar in shape to that of Fig. 9. The curve for the movement in space of Müller's organ is similar, though the amplitude of movement is less, particularly at high frequencies. When analysis is made of the relative movement between the folded body (point *T*) and the point on Müller's organ (*M*) the following points emerge: because there is always a substantial phase difference in excess of 60° between the two vibrations, the resultant strain is of larger amplitude than that of either of the two components; because the phase of the vibration of the folded body with respect to that of the vertical vibration of Müller's organ slides from −65° at 2 kHz to −150° at 5 kHz, there is amplification of the tympanal vibration which is greater at high frequencies.

It is thus impossible, from a study of the tympanum alone, to predict the probable frequency response of the system. This point will be re-emphasized in section D iii, which examines the effect of removal of Müller's organ. The amplitude of vibration of Müller's organ falls steadily with respect to that of the tympanum from 2 to 5 kHz. The phase angle between the peak inward strain of the tympanum and the peak strain between points *T* and *M* slides from −125° at 2 kHz to −180° at 5 kHz (Fig. 12 B). These two points suggest that there is a damped resonance of Müller's organ on the tympanum below 2 kHz.

In other experiments we measured and compared the strains along different axes and between different parts of the ear. Two typical results are shown in Fig. 13. In Fig. 13 A it is shown that the strains observed along different axes can be similar at certain frequencies and differ by a factor of two (6 dB) at other frequencies. In Fig. 13 B it can be seen that, measuring along axes parallel to the plane of the tympanum but at different distances from it, different frequency response curves are obtained for the strain between points on sclerites and on Müller's organ. The curves were obtained by measuring on the outside of the sclerites and Müller's organ. The resulting strain in the sensory dendrites may be very different from what we have observed as it is hard to be sure, from our anatomical studies and those published elsewhere, of the exact orientation of the dendrites.

We have observed a number of different strain/frequency spectra for other positions and directions of Müller's organ. These emphasize that there is a wide range in the frequency dependence of the possible 'adequate stimulus' available to drive sensory cells situated in different parts of Müller's organ and that there is unlikely to be a unique frequency response of say, the *a* cells. As a further complication, we have noted that at some points and frequencies the strain oscillates symmetrically about the equilibrium

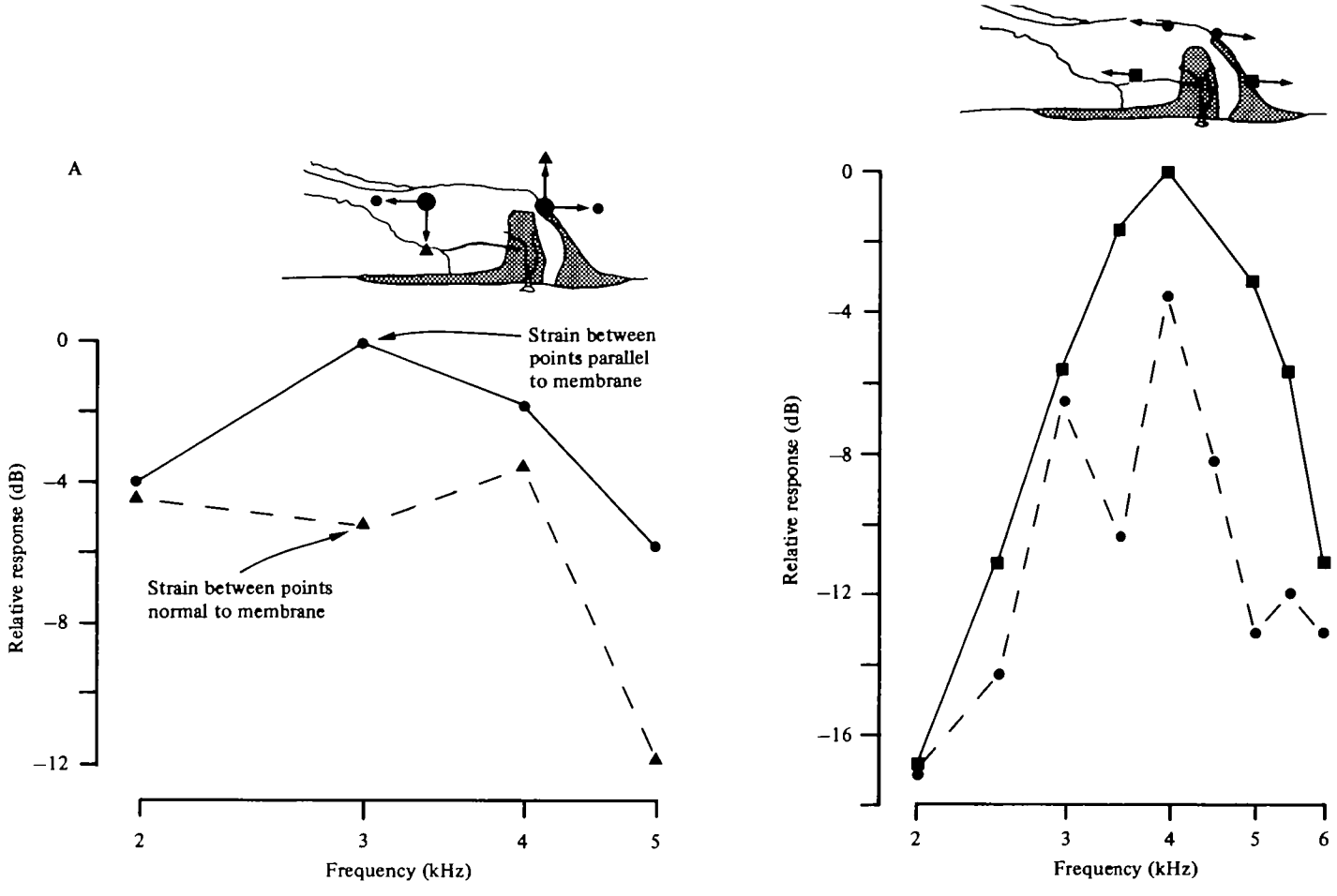

Fig. 13. Plots of relative amplitude of strain between points on sclerites and points on Müller's organ, against frequency. The sound pressure was a constant 122 dB rms.

(A) Showing the difference in the observed strain along axes parallel to the tympanum (●—●) and normal to the tympanum (▲- - -▲). The axes along which the measurements were made and the positions of the points are shown in the inset. Relative response is referred to the maximum strain, which was 53 μm peak-to-peak.

(B) Showing the strain between pairs of points on different parts of the same ear, as indicated in the inset. The relative response is referred to the maximum strain observed for the lower pair of points (■—■), which was 27 μm peak-to-peak.

position (e.g. Fig. 10, point B) but that in other cases there is a shift in the centre of oscillation so as to impose both a standing strain and an oscillating strain on the sensory cell (e.g. Fig. 10, points G and H). As it is not known whether a phasic oscillation or a tonic d.c. strain acts as an adequate stimulus to the sensory cells or whether the cells are velocity, amplitude or force transducers, there is little point in attempting to make further predictions about the neural frequency response of the system.

(iii) *Effect of removal of Muller's organ*

The mass of Müller's organ is similar to that of the thin membrane (Table 1). Even though its coupling to the tympanum is highly compliant, it is driven into oscillation by the vibration of the tympanum.

On the same ear as that described in Fig. 12, a set of measurements was made between 2 and 5 kHz on the folded body, after which Müller's organ was removed with forceps and a new set of measurements was made. The results are shown in Figs. 14 A, B. After removal of Müller's organ, the amplitude of vibration of the tympanum increases by over 4 dB though there is little change in the resonant frequency. The Q of the resonance (see Appendix), shown either by the -3 dB bandwidth (Fig. 14 A) or the $-45°$ to $-135°$ bandwidth (Fig. 14B), changes from about 3·6 in the intact ear to about 8 after removal of Müller's organ. This shows that the major interaction of Müller's organ with the tympanum at this frequency is viscous (or resistive) damping, corroborating the evidence presented in Results D ii, Fig. 12. This is in marked contrast to the effect of adding a mass load to the tympanum (Results D iv), which shifts the resonant frequency but does not appreciably alter the damping. The static compliance measurements on Müller's organ showed that there was a weak elastic coupling between it and the folded body; the present measurements confirm that there is an additional strong resistive coupling (see Discussion, section C). Since we are looking at Müller's organ above its resonance, its motion will be controlled by its mass and the resistance of its drive. The effect of a viscous coupling increases by 6 dB per octave while the amplitude of mass-limited systems decreases by 12 dB per octave. Overall, the amplitude of vibration of Müller's organ in space should decay by 6 dB per octave with respect to its drive; this can be seen in Fig. 12.

The vibration of the styliform plate, before and after removal of Müller's organ, has also been examined. There is only a slight effect on the vibration between 4 and 5 kHz, but the amplitude of vibration at low frequencies is appreciably reduced. This suggests that some of the observed coupling between the folded body and the styliform plate (Fig. 9) occurs through Müller's organ.

(iv) *The effect of loading the tympanum*

A piece of wire weighing approximately 10 μg was attached to the folded body on the external surface of the tympanum using silicone grease. A frequency/amplitude and phase scan of the vibration was then made, the piece of wire was removed (but not all the grease), and the scan was repeated. There was no evidence that the piece of wire had shifted. The results are shown in Fig. 15. It will be seen that the load lowered the primary resonant frequency, at which the phase lag was 90°, from 2·5 down to 2 kHz, and also shifted the 4·5 kHz resonance to a lower value. For the primary

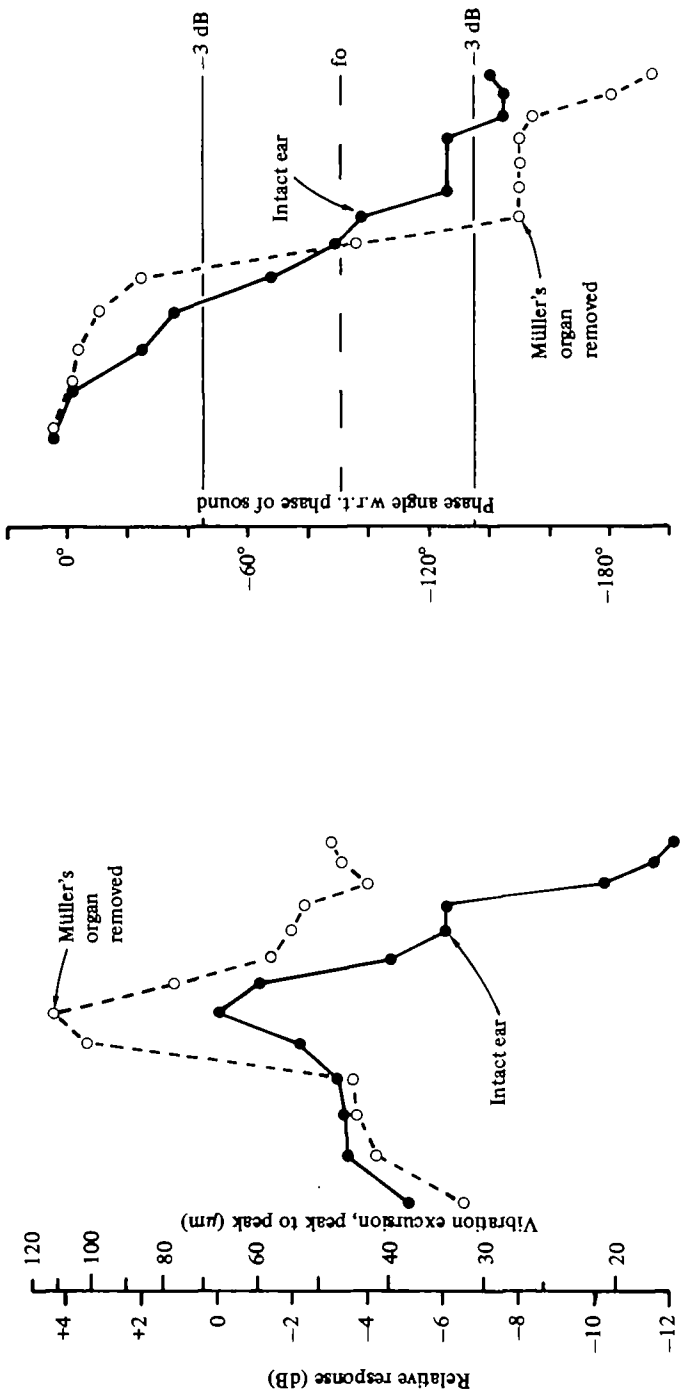

Fig. 14. Graphs of the vibration normal to the tympanum of a point on the folded body (point *T* of Fig. 12) driven by a sound of 122 dB between 2 and 5 kHz measured on the same ear before and after removal of Müller's organ.

(A) Plot of the amplitude of vibration against frequency. Relative amplitude is expressed in dB with respect to peak amplitude observed for the intact ear.

(B) Plot of phase angle of the vibration against frequency; the use of the horizontal lines at $-45°$, $-90°$ and $-135°$ is described in the Appendix and is discussed in Results D iii.

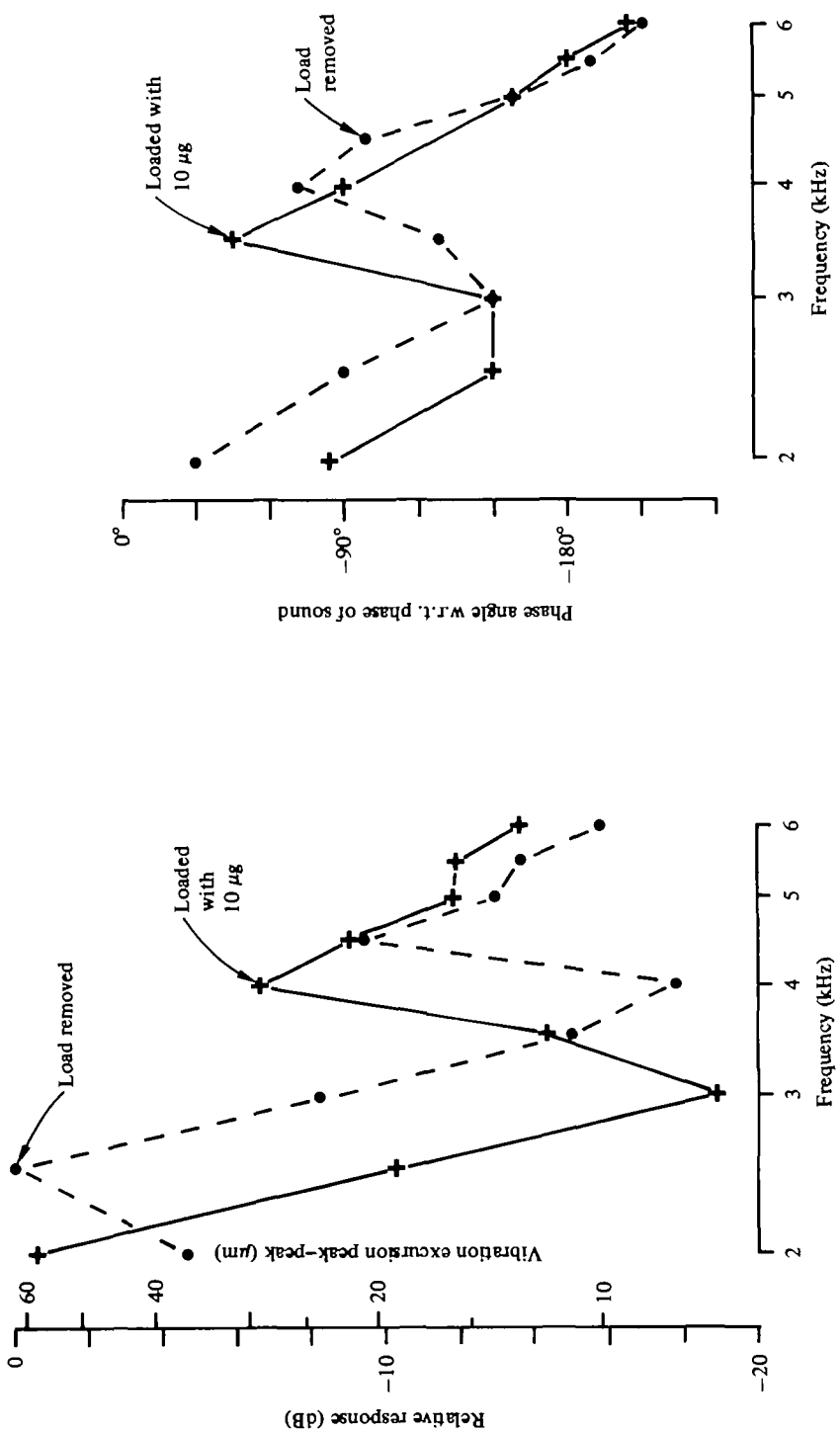

Fig. 15. The effect of loading the tympanum. Graphs of the vibration after attaching a 10 μm bent wire weight to the folded body with grease (point *A*, Fig. 8) and after removal of the weight. The same ear was used for both sets of measurements, Müller's organ was left intact and the sound pressure was 122 dB rms over the range 2–6 kHz.
(A) Plot of vibration amplitude *vs.* frequency; relative amplitude is plotted in dB with respect to peak amplitude after removal of the load.
(B) Plot of phase of the vibration with respect to phase of the driving sound.

resonance there is little evidence of any change in the sharpness of the response curve, which suggests that the added load acted as a pure mass as expected.

From equation 1 (Discussion, section B) it can be calculated that the effective mass of tympanum that shifts in resonant frequency from 2·5 to 2 kHz with the addition of a 10 μg mass is 18 μg; this value is likely to be an overestimate because of uncertainties in the mass of the grease used to hold the wire load in place.

We cannot place much value of this experiment for measuring the effective mass of the tympanum, though it is of value in showing the difference between the effect on the vibration of the membrane of a rigidly coupled mass and the visco-elastic coupling of the similar mass of Müller's organ.

(v) *Behaviour at frequencies above 7 kHz*

Because of uncertainties in the performance of the high-sound-pressure closed-field source above 7 kHz, we have been unable to make measurements of either tympanal vibrations or strains in Müller's organ in this range. By using other sources which gave an output at frequencies up to 14 kHz, we have made observations over this frequency band.

The amplitude of vibration of both the styliform plate and the folded body fall continuously, and the rocking and squeezing strains of Müller's organ also diminish. At frequencies between 10 and 12 kHz, the pyriform vesicle can be seen to vibrate and this, in turn, sets up a squeezing motion in the middle of the fusiform body in the region of the sensory dendrites of the *d* cells (Fig. 5). We have not been able to produce a tuning curve or to measure the phase relations of this strain.

The behaviour of the tympanum at these frequencies is compatible with our earlier proposals that the larger sclerites will behave above resonance as mass-limited systems, which will then permit the lighter, thinner region of the tympanum surrounding the pyriform vesicle to vibrate at its own resonant frequency. Michelsen (1971*a*) shows response curves for the *d* cells with sensitivity peaks between 10 and 20 kHz, which agrees with our observations. His holograms at 9·5 and 12 kHz show an antinode of vibration near the pyriform vesicle (Michelsen, 1971*b*).

DISCUSSION

(A) *The acoustic input*

The driving force across the locust tympanum depends upon the sound pressure difference across it. This is dependent on the way in which the ear is mounted. The experimental conditions used by and reviewed by Michelsen (1971*a*, *b* and *c*) differ from those used here, so a comment on the differences in the expected driving forces is desirable.

In the 'isolated ear' the tympanum in its cuticular rim is mounted normal to the soundwave. In the 'intact ear' the tympanum of one side is separated by air sacs from the tympanum on the opposite side. In both cases, interaction between sound incident on the inner and outer tympanal surfaces will affect the net driving force in a frequency dependent manner. There are also other frequency-dependent effects, the radiation resistance of the tympanum and, in the 'intact ear', diffraction by the body; both of

these, for a given sound pressure, will tend to cause the driving force to rise with increasing frequency.

In our closed-field situation, the effective driving force on one side of the tympanum is close to the product of the sound pressure and the area of the vibrating structure, modified by the re-radiation from both sides of the tympanum which, because the radiation impedance of the tympanum is small, will have a trivial effect. The closed-field set-up provides a driving force which depends mainly on the impedance of the source, whereas the free-field situation provides a driving force which depends largely on the load due to the impedance of the tympanum. In the closed field, therefore, the force will depend largely on the frequency response of the source, but in the free field the force will depend on the frequency response of both source and load, so the force will tend to rise with frequency at a constant sound pressure as the impedance of the tympanum rises.

It follows that our amplitude–frequency plots cannot be used to predict the frequency response of the ear of an intact locust in the free field with the same *apparent* sound pressure. With appropriate corrections for the relative impedances of the source and the ear in free and closed fields, such a prediction could be made but, from the discussion in Michelsen (1971*c*), there would be considerable uncertainty. Despite this, it is certain that in the free field the change in driving force with frequency will act in the opposite sense to the effect of the mass of the tympanum. The decreasing amplitudes of vibration that we observe above resonance with a more or less constant driving force will be modified and even compensated in the free field by the increase in driving force with frequency that occurs in the free field.

(B) *The predicted resonant frequencies of the tympanum*

The uneven distribution of the mass and stiffness of the tympanum, which has not previously been observed, suggests that the membrane is designed so that its different regions can vibrate relatively independently and at resonant frequencies determined by their mass and compliance. Any calculations based on this premise will be imprecise because estimates of the areas involved, their masses and their compliances are all subject to uncertainty.

The resonant frequency, f_0, of a simple mass suspended on a spring is given by:

$$f_0 = \frac{1}{2\pi}\sqrt{\frac{1}{m.c_m}}, \qquad (1a)$$

where m is the mass and c_m is the compliance.

The use of this equation is justified if the different parts of the membrane behave as separate rigid pistons. Since the peripheral parts of the regions may move with a different amplitude, the apparent mass and compliance may differ from those measured. An appropriate correction, assuming that the membrane is homogeneous and tensed, can be introduced into Equation 1*a* which then becomes:

$$f_0 = 0{\cdot}383\sqrt{\frac{1}{8m.c_m}}. \qquad (1b)$$

Table 4. *Calculated resonant frequency (f_0) of different regions of the locust ear. Values are calculated from data in Tables 1 and 2*

Region (see Fig. 6)	Mass (μg)	Compliance ($m.N^{-1}$)	Resonant frequency f_0 (kHz) From equation 1 *a*	From equation 1 *b*
Müller's organ	10	7·5	0·58	—
Folded body (2) and surround	3·9	0·38	4·1	3·5
Styliform foot and surround (3)	2·9	0·21	6·5	5·6
Pyriform vesicle and surround (4)	0·32	0·22	19	16·5

This, derived from Morse (1948) is a transposition of that used by Michelsen (1971*b*, Equation 3). When it is applied to the different regions of the membrane, the values calculated for f_0 are 0·85 times those obtained from using Equation 1*a*. The values obtained using both equations are shown in Table 4.

The calculated values of the resonant frequency of the folded body region (3·5–4·1 kHz) and the styliform body region (5·6–6·5 kHz) are similar to those observed in our examination of the tympanal vibration (Results C ii). We do not have measurements of the vibration of the area around the pyriform vesicle, but its independent vibration at around 12 kHz has been observed.

The behaviour of the tympanum will be complicated by the coupling to and damping of one region by another. The thin membrane appears to have a rather higher resonant frequency than the folded body and styliform body regions. The motion of the thin membrane will be damped by its internal resistance and, above their resonances, reduced by the reduced amplitude of the mass-limited regions around the sclerites. The regions around the sclerites are affected by internal damping of the cuticle and by that due to Müller's organ.

Because of its very high compliance on the tympanum and relatively large mass, the major loading of the tympanum by Müller's organ above its resonant frequency will be viscous, and so its presence will affect the Q of the resonances and the coupling between them significantly but only have a minor effect on the resonant frequencies. This has been seen in Results D iii.

Even though there is clear evidence of interaction between the different parts of the ear, it seems a useful simplification to regard the different regions detailed in Table 4 as if they are independent oscillators, each of which will show a large amplitude of motion at resonance, falling off more rapidly at the high-frequency side, which is mass-limited, than on the low frequency side which is stiffness-limited. These effects can be seen in Michelsen's (1971*b*) holograms.

(C) *Transfer of energy from the tympanum to Muller's organ*

When Müller's organ is removed, the Q of the resonance rises from 3·6 in the intact ear to 8. This is due to reduction in the damping. The magnitude of the damping resistance can be calculated in terms of the mass, m, and the Q of the system (Morse, 1948). The damping resistance, R_m, in $kg.s^{-1}$ or $N.s.m^{-1}$, is given by:

$$R_m = \frac{2\pi . f_0 . m}{Q}, \tag{2}$$

Table 5. *Energetics of the motion of the tympanum and Müller's organ for an ear driven by 122 dB rms sound*

(A)	Mass of folded body region	4×10^{-9} kg	
	Mass of Müller's organ	10×10^{-9} kg	
(B) Ear kinematics		rms amplitude in space	rms velocity at 3·25 kHz (equation 3)
	Folded body, intact ear	25×10^{-6} m	0·51 m.s^{-1}
	Folded body, no. m.o.	39×10^{-6} m	0·8 m.s^{-1}
	Müller's organ	18×10^{-6} m	0·36 m.s^{-1}
		Q (Equation A1)	Damping resistance (Equation 2)
(C) Damping of ear			
	Folded body, intact ear	3·6	23×10^{-6} kg.s^{-1}
	Folded body, no m.o.	8·0	10×10^{-6} kg.s^{-1}
	Damping due to connexions of Müller's organ	—	13×10^{-6} kg.s^{-1}
(D) Power dissipated, calculated from resistance and velocity (Equations 3 and 4)			
	Folded body, intact	6×10^{-6} W	
	Folded body, no m.o.	$6{\cdot}4 \times 10^{-6}$ W	
	In Müller's organ, intact ear	$3{\cdot}4 \times 10^{-6}$ W	

where f_0 is the resonant frequency in Hz. This calculation can be made first for the intact ear and then after removal of Müller's organ using the data in Figs. 12 and 14. Because the load due to Müller's organ is resistive, as shown by the 90° phase angle between the motion of the tympanum and that of Müller's organ and the lack of change of resonant frequency on removal of Müller's organ, it is legitimate to derive the resistance due to Müller's organ by simple subtraction. This partitions the resistance of the ear as is shown in Table 5.

The power dissipated in the tympanum and Müller's organ can now be calculated from the resistance and velocity of the vibrations. Velocity, in m.s^{-1}, for a harmonic vibration is given by:

$$\text{rms velocity} = \frac{\text{amplitude} \times 2\pi . f_0}{\sqrt{2}}. \tag{3}$$

Calculated values are given in Table 5.
Power, in W, then is given by:

$$\text{power} = (\text{rms velocity})^2 \times \text{resistance}. \tag{4}$$

The values obtained (Table 5) for the intact ear and the tympanum after removal of Müller's organ are closely similar because the sound power input is the same. The power dissipated in the resistance of Müller's organ is about half that needed to sustain the motion of the intact ear.

The tympanum can be regarded as the source and Müller's organ can be regarded as the load of a mechanical circuit. For optimal transfer of power from source to load, the load should be resistive and its resistance should equal that of the source. These conditions are approached (Table 5).

The tympanum, in turn, should be coupled to the driving sound pressure so as to optimize the transfer of power. For this purpose, ideally, the whole tympanum should act as a rigid piston so that the whole surface area is usable, it should be as light as possible and only be damped by the sensory cells. This will result in a single serie

of resonances, and frequency analysis will have to be performed in an accessory structure. If, as appears to occur in locusts, frequency analysis results from comparison between frequency-dependent differential vibration of adjacent regions of the tympanum, the coupling between different parts of the tympanum should be such that the lighter parts augment the vibration of the heavier parts but the heavier parts do not impede the vibration of the lighter parts.

(D) *The mechanics of frequency analysis*

Though Michelsen (1973) comments that 'the possibility remains that the Müller organ is not just a passive observer of the tympanal vibrations, but that it plays an active role in the transduction process' this role has been insufficiently studied. We have shown that the mass, stiffness and damping of Müller's organ modify the vibration of the tympanal membrane. We also show that because the motion of the organ differs from that of the adjacent regions of the membrane, strains occur in the regions of attachment of the organ on to the tympanum. Such strains could provide adequate mechanical stimuli.

Differences in the phase and amplitude of the vibrations of the folded body and the styliform body produce motions acting roughly normal to each other that behave as drives to Müller's organ. Such drives can produce Lissajous' figures which characteristically vary in shape according to the relative phase and magnitude of the two drives (see, e.g. Rider & Uslam, 1959). The advantage of such an arrangement in the locust ear is unclear, particularly as it would appear at first sight that the two drives will interact to reduce the sharpness of tuning of each other by viscous coupling through Müller's organ.

When the amplitude of a Lissajous' figure is measured along either drive axis, the frequency response curve obtained is that of the system to that particular drive. The frequency response curve along a third axis lying between the two drive axes will be different from that along either drive axis and will depend on the inclination of the third axis to the two drive axes.

The receptor cells attached to the folded body (*c* cells), elevated process (*a* cells) and styliform body (*b* cells) run roughly normal, at 45° and parallel to the plane of the tympanum, respectively. Given that there are *two* (coupled) oscillators with different resonant frequencies driving the motion of Müller's organ, this anatomical arrangement can be expected to generate *three* different classes of receptor frequency response curves. From the work of Michelsen on *Schistocerca* (1971*a*) and Römer on *Locusta* (1976), it appears that these three groups of cells have peak sensitivities at different frequencies. For *Locusta*, where the response spectra of single cells were recorded from the axons, peak of sensitivity occurs at about 3·5 kHz, 4 kHz and 6 kHz. (and 12–15 kHz, presumably from the *d* cells).

Though limited frequency analysis is possible using only two receptor response curves, ambiguities occur near the sensitivity peaks of each type of receptor. This is well known in human colour vision, where deficiencies in either the 'red' or the 'green' receptor cause confusion *both* in the red or green *and* in the blue, where the ability to discriminate between light of different colour becomes impaired or even impossible. When all three colour receptors are present, colour discrimination is

possible over a band which extends from above the wavelength of peak sensitivity of the 'red' receptor to below the wavelength of peak sensitivity of the 'blue' receptor (for review see Marriott, 1962). A similar problem appears to exist in locust hearing, but here it appears that the third frequency response curve that is required for frequency analysis over a broad band is generated mechanically.

The role of the *d* cells, on this argument, is to extend the range of hearing to high frequencies but, from their apparent broad-band response, to be of little importance in frequency analysis above 10–15 kHz.

(E) *Acridiid song and hearing*

The songs of most acridiids consist of impulses produced by the passage of a toothed cuticular ridge over another ridge. The resulting song has a broad frequency spectrum and shows rapid changes in amplitude. The impulse rate of such songs has been shown by Skovmand & Pedersen (1978) to be an important character. The locust ear appears to be capable of analysing the rapid changes in frequency as the Q is low (Michelsen 1971*b* and this work). In the locust ear all the receptors are driven by a common tympanum so the initial responses of different cell groups will have predictable phase and temporal relations to each other. The ear is thus well suited to the analysis of acoustic impulses.

(F) *General conclusion*

The present work confirms that the basis for frequency analysis in the locust ear is the differential vibration of different parts of the tympanum, which leads to differential adequate stimuli to different receptors in Müller's organ. Since the function of the sensory cells is to transduce energy, it is desirable that they should act resistively and that the tuning should be in the accessory structures. Our study highlights the importance of the transduction mechanism in the study of the frequency response of sensory systems.

APPENDIX

Conventions used in description of resonant systems

The motion of the system is not usually in phase with the force, the angle of lag of the displacement being given by the phase angle, which approaches zero when frequency is well below resonance, is $\pi/2$ or 90° at the resonant frequency and approaches π or 180° when the frequency is far above resonance (indicating that the displacement is opposite to the force). This convention is used here and follows Morse (1948), p. 31.

At resonance, the amplitude of vibration of a driven resonant system is maximal. Below resonance, depending on the driving conditions, the amplitude of vibration tends to be constant because the system is then limited by stiffness or its analogue, which is independent of frequency. Above resonance, the amplitude of vibration falls continuously because the system is then limited by the mass or its analogue, which has a frequency-dependent effect.

At resonance, the amplitude of the vibration is controlled by the viscosity or damp-

ing of the system. A simple measure of this is Q or the Quality Factor, which is given by

$$Q = \frac{\text{bandwidth at 3 dB below peak}}{\text{resonant frequency, } f_0} \qquad \text{(A1}a\text{)}$$

or, because at the -3 dB points, the tangent of the phase angle is 1,

$$Q = \frac{\text{bandwidth at } \pm 45^\circ \text{ with respect to phase at resonance}}{\text{resonant frequency, } f_0} \qquad \text{(A1}b\text{)}$$

It is often easier to specify the phase in a system than to specify the amplitude. For this reason, it may be a good and simple indication of the sharpness of tuning or Q to examine the slope of the phase *vs.* frequency plot. We therefore make use of this indication in Figs. 12B and 14B.

In damped or natural resonance, the frequency at which maximum amplitude is observed is different from the resonant frequency calculated from the mass and stiffness of the system. For a Q of 3, the natural resonant frequency is (0·986 × resonant frequency). As such a small difference cannot be detected in our experiments, we have ignored this.

For a fuller discussion of the properties of simple resonant systems see either Langford-Smith (1957, chap. 9) or Morse (1948).

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