On the pressure of the endolymphatic, the perilymphatic, and the cerebrospinal fluid, with data on the endolymphatic membranes

Experiments on cats and guinea pigs

B.I.J. Beentjes

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The work for this thesis was carried out in the department of otorhinolaryngology of the University of Amsterdam (Head: Prof. Dr. L. B. W. Jongkees)

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To the memory of my father
To those I love

CONTENTS

Chapter I

INTRODUCTION

In 1861 Prosper Ménière showed that the labyrinth was the centre of a certain pattern of signs and symptoms which until then were often diagnosed as 'apoplectiform cerebral congestion'.

Knapp (1871) was the first otologist who mentioned an intralabyrinthine pressure increase as the cause of a Ménière attack. He suggested that perhaps glaucoma of the eye was the counterpart of Ménière's disease. This suggestion was supported by the discovery of Hallpike and Cairns (1938). They reported that a dilatation of the endolymphatic labyrinth was present in two cases of 'Ménière's syndrome' which terminated fatally after intracranial vestibular nerve section. This idiopathic type of hydrops has since been confirmed by a great number of investigations in connection with Ménière's disease, which have been reviewed by Altmann and Kornfeld up to 1965. There have been only a few reports, reviewed by Wustrow and Borkowsky (1960), of patients suffering from Ménière's disease in which the subsequent histological examination failed to demonstrate endolymphatic hydrops. The diagnoses in these cases have been questioned (Kristensen 1961). It is commonly accepted that the histopathological picture of the labyrinth in Ménière's disease consists in the enlargement of the endolymphatic compartment at the cost of the perilymphatic one and we may say that the concept of a hydropic labyrinth is well established in Ménière's disease.

The distension of the endolymphatic compartment is generally considered an essential clue which may explain the etiology and pathogenesis of Ménière's disease. Some investigators hold the cause of Ménière's disease and hydrops to be identical. Thus, it is not surprising that hydrops is often taken as the starting point when looking for an explanation of the signs and symptoms of Ménière's disease.

The histopathological picture in Ménière's disease made experts speak of endolymphatic hypertension. Certainly, the alteration in the histological picture suggests to have been caused by a pressure increase in the endolymphatic space relative to the pressure of the perilymph. The assumption that an equal pressure exists in both perilymph and endolymph is probably rooted in the normal histological picture. House stated in 1967 at an international symposium on Ménière's disease held in Rochester (Minnesota), that equal endolymph and perilymph pressures are necessary for normal cochlear and vestibular function.

The outcome of extensive experimental work on the guinea pig (Weille et al. 1958, 1961, Martinez 1969) contradicted this assumption entirely;

their investigations showed a higher perilymphatic pressure. Martinez measured an average pressure difference of 2.8 mm Hg in the guinea pig during anaphylactic shock. His publication of a histological examination of a shocked animal's cochlea showed only a minute dilatation of the scala media, if any. Some questions arise: What difference in pressure would be necessary to produce a histological picture as seen in Ménière's disease? Is the endolymphatic pressure in Ménière's disease increased to such an extent that hearing loss would result by such a mechanism as compression of the hair cells (MYGIND 1950, DEDERDING 1929, CAWTHORNE 1947), or are smaller vessels compressed causing anoxia of sensory nerve cells? What pressure difference is needed for rupture of the endolymphatic compartment by tearing of Reissner's membrane, as some say occurs in Ménière's disease? (LAWRENCE and MCCABE 1959, SCHUKNECHT 1960, 1963). To our knowledge only HENRIKSSON et al. (1966) have measured the positive pressure gradient between endolymph and perilymph in the frog at which rupture occurs; a gradient of 5 cm or more water was needed and the rupture mostly involved the sacculus. No such investigation has been performed in mammals. For anatomical and histological reasons these animals are preferred, so as to be able to relate the results to man. (The frog, for instance, has no cochlea.)

We performed a great number of experiments concerning intralabyrinthine pressure phenomena, also in relation to the cerebrospinal fluid pressure.

First of all we tried to resolve the apparent controversy regarding the pressure gradient between endolymph and perilymph. We made a critical analysis of the gigantic work of Weille c.s. and Martinez by reproducing analogous experiments on a moderate scale. Changing the approach to the endolabyrinthine fluids, we performed experiments on cats in order to check whether a pressure difference could be established. A special technique had to be developed for this purpose. Not only the correlation between the pressures of the cerebrospinal fluid and the perilymph (cf KERTH and ALLEN 1963), but also between the pressures of the cerebrospinal fluid and the endolymph was investigated.

Finally, we performed experiments regarding the pressure gradient necessary to cause rupture of the endolymphatic compartment. Some interesting phenomena occurred in this part of our study.

The knowledge of Ménière's disease is still rather meagre, as is illustrated by the variety of treatments which aim to suppress the symptoms, and are not well enough developed yet to really cure the cause. We have investigated only a few aspects of the labyrinth in an effort to provide some pieces for the puzzle of etiology and pathogenesis of Ménière's disease from which still so many pieces are missing.

CHAPTER II

A CRITICAL ANALYSIS OF THE ENDOLYMPHATIC AND THE PERILYMPHATIC PRESSURE AS MEASURED ACCORDING TO THE LITERATURE

WEILLE and coworkers (1958, 1961) and MARTINEZ (1969) have reported extensive results regarding the pressure of the perilymph as related to that of the endolymph; these investigators concluded from their experimental work which took them about five years, that in a living guinea pig the perilymphatic pressure exceeds the endolymphatic one. This concept can be found in several handbooks. Undoubtedly, the guinea pig served as their experimental animal since its stria vascularis is pigmented and distinguishable. In all their endolymphatic measurements they tried to reach the endolymph via the bony cochlear wall using the darkened area as a landmark. In virtually all of their perilymphatic measurements they approached the perilymph via a spot just beside this mark, the mark itself being carefully avoided. In 21 pressuregrams of WEILLE c.s. (1958) the perilymph was reached via the secondary tympanic membrane.

Using 2,100 guinea pigs WEILLE et al. (1958, 1961) obtained 908 pressuregrams, 175 of which were perilymphatic and 733 endolymphatic measurements. The values they found for the perilymphatic pressure varied from 2 up to 93 mm Hg versus a range of from 1 up to 39 mm Hg for the endolymphatic pressure.

Unlike Weille et al., Martinez performed simultaneous pressure measurements of perilymph and endolymph. The number of guinea pigs used in this part of his experiments is not clear. He reports 35% of all his experiments, totaling 1,300, to be successful. According to his publication the endolymphatic pressure ranges from 1.3–3.2 mm Hg, whilst the perilymphatic pressure reportedly ranges from 2.2–6.6 mm Hg.

At first we used the same approach to the labyrinthine fluids, proceeding in the footsteps of these recognized predecessors. However, a frustration lasting almost one year, in which some 300 guinea pigs were sacrificed, forced us to admit that in our hands no decent results could be achieved in this way. Our data up to then were too unreliable to be conclusive. This experience and a scrutiny of the publications of Weille et al. and Martinez made us doubt the efficacy of the method and caused us to search for a better way of pressure measurement; WEILLE et al. (1958) had tried the route of the secondary tympanic membrane in order to measure the perilymphatic pressure in 100 guinea pigs; they reportedly encountered severe problems of leakage along the measuring microcannula.

In 25 experiments which we performed ourselves we found leakage without fail. To overcome this difficulty we developed a special technique which later on we perfectioned to the point that we could also measure the endolymphatic pressure via the basilar membrane, without mixing perilymph and endolymph along the measuring microcannula. Chapter III presents a detailed acccount of how this was achieved and of the outcome; in this chapter we will refer to some of the results in anticipation.

Based on the publications of Weille et al. and of Martinez and on our own experience we will now go into a critical analysis of several difficulties inherent to the conventional method, mainly as an effort to explain why Weille et al. and Martinez found the perilymphatic pressure to be higher than the endolymphatic one:

Firstly, there is the feature of occlusion; Weille et al. employed a hand-drill technique to form microfenestrae in the bony cochlear wall, while Martinez utilized a micromanipulator for thinning down the bone overlying the compartments of interest. Martinez states that, while drilling the microfenestrae, he took care not to penetrate into the scalae in order to avoid fluid leakage. We could imagine that in drilling the microfenestrae fluid leakage could indeed be avoided, although in practice we have repeatedly found that the hole we had drilled was filled with fluid in spite of the usage of a micromanipulator. From Martinez's statement we conclude that at least the spiral ligament must have been intact at the end of the drilling stage, which conclusion finds confirmation further on in his publication (MARTINEZ 1969). Therefore, the minimal thickness left in the endosteal layer, which Martinez says to have broken through with the measuring microcannula immediately preceeding endolymphatic pressure measurement, includes the spiral ligament. Consequently, in order to reach the endolymphatic space, the tip of the microcannula has to pierce a complete capillary meshwork as described by SMITH (1951, 1957). Also, WEILLE et al. (1954, 1958, 1961) tried to avoid leakage and aimed at not injuring the underlying spiral ligament in the process of drilling. Weille et al. and Martinez found that the lumen of the microcannula can be obstructed in the act of piercing the endosteal layer. In a large portion of our own measurements we found congestion of the microcannula in reaching the endolabyrinthine space when we employed the method just mentioned. By congestion we do not necessarily mean occlusion, but also throttle. The latter type of congestion may result in retardation of establishing pressure between the fluid under consideration and the measuring device. The occurrence and severity of the congestion are definitely more pronounced when the endolymphatic pressure is involved than when one is investigating the perilymphatic pressure; this is not surprising if one realises that the thickness of the endosteal layer which has to be perforated by the microcannula tip is markedly different for peri- and endolymph. During the transition of pressure buildup (see chapter III, page 23) a throttling effect may, we feel, turn into an occlu-

sion due to the presence of loose material in the space of interest. The details of how exactly we deduced the degree of congestion will be described in chapter III. In our experiments the pressure buildup was frequently slow, especially if endolymphatic measurements were performed; Martinez reports that the pressure buildup for the endolymph is particularly slow and he uses this as a feature to judge whether he has reached the endolymphatic space. In our opinion this reasoning is not correct and the rate of pressure buildup should not be more than a mere indication of the degree of congestion. If one plots the data of the three sample experiments of MARTINEZ (1969, pp. 38, 39 and 40) during anaphylactic shock our hypothesis is confirmed. MARTINEZ's statement (1969, p. 37) that 'when one pipet was opened to atmosphere, the output meter belonging to the other pipet showed no change', should be interpreted, we feel, as a case of occlusion in preference over an unruptured endolymphatic compartment.

Secondly, it is hard to rule out the possibility of leakage due to lack of a perfect seal between the microcannula and the bone, particularly in the pressure measurement of the endolymph, as there is an inclination to drive the microcannula tip through the microfenestra too gingerly out of fear to otherwise damage Reissner's membrane; the conical front of the microcannula then certainly lacks in serving as a tight seal.

Thirdly, in the effort to measure endolymphatic pressure the possibility exists that the microcannula tip has torn away the spiral ligament or has not pierced it (WEILLE et al. 1958). This also leads to excessively low values for the endolymphatic pressures.

The reasons quoted above could have invoked the idea that the perilymphatic pressure exceeds the endolymphatic one if such pressures are measured in a way as described in the foregoing exposition.

Many difficulties shown in the measurement of the perilymphatic and the endolymphatic pressure have been pointed out by Martinez and partly mentioned by Weille et al. Some of the difficulties we would like to discuss in more detail:

In order to determine the value of the absolute pressure, the pressure transducer has to be placed meticulously on a level equal to that of the fluid of interest. Neglect of this requirement could explain the wide range of the values found by Weille et al.

When one anesthetizes a guinea pig with sodium pentobarbital by intraperitoneal injection, the vasolability of this animal is manifest. Martinez quotes average values for the 'pre-shock' endolymphatic and perilymphatic pressures being 2.0 and 3.5 mm Hg respectively, the arterial pre-shock pressure equalizing 28.6 mm Hg; the normal arterial pressure in the guinea pig has been reported to be on average 81–90 mm Hg (SPECTOR 1956).

Ascertaining that the tip of the microcannula, being guided by the pigmented area of the stria vascularis, reaches the right place, is extremely

difficult. WEILLE et al. (1961), for instance, state that in a series of 59 experiments aimed at measuring endolymphatic pressure, histological examination revealed that in 23 cases the microcannula had entered the scala vestibuli or scala tympani. Consequently it is necessary to check whether the microcannula has reached the right site in some way or another. In this way Martinez's proof of reaching the desired space assumes what should be concluded and concludes what should be assumed.

By simultaneously measuring the pressure of both the perilymph and endolymph, as performed by Martinez, one circumvents some difficulties which is meritorious; for example, the biological variations between individual members of one species, in this case the guinea pig, become unimportant if the endolymphatic pressure is compared with the perilymphatic one in the same individual. On the other hand, in simultaneous measurements of the perilymphatic and the endolymphatic pressure, two measuring systems are required. The construction of two measuring systems displaying identical characteristics under the various actual operating conditions is next to impossible. Therefore, detection of small differences in pressure values appears to be exceedingly difficult. By measuring perilymphatic and endolymphatic pressures sequentially and in one throw with the same detector, we found this difficulty to vanish. We employed this method.

The major portion of the remaining difficulties we avoided, as already mentioned, by measuring the perilymphatic pressure after having inserted the tip of the microcannula via the secondary tympanic membrane and subsequently measuring the endolymphatic pressure after piercing the basilar membrane with the same microcannula tip. This approach towards the endolabyrinthine fluids for pressure measurements no longer favours the guinea pig as the experimental animal. Therefore, most of our further measurements have been carried out on cats. We appreciate the huge amount of work performed by both Weille et al. and Martinez in their measurements of endolabyrinthine fluids, but it is our opinion that the difficulties in measuring the perilymphatic and the endolymphatic pressure via the cochlear wall cannot be overcome.

Chapter III

SEQUENTIAL MEASUREMENT OF THE PERILYMPHATIC AND THE ENDOLYMPHATIC PRESSURE BY A NEW APPROACH

In the previous chapter doubts were expressed about the suitability of the bony cochlear wall as the optimal site to enter the endolabyrinthine compartment for pressure measurements. The round window seemed a better spot, provided the problems of leakage along the measuring microcannula could be solved.

We have also indicated in chapter II that sequential measurement with one single measuring system is almost a necessity when small pressure differences need to be detected.

In this chapter we will limit ourselves to the two compartment system of peri- and endolymph, and describe how in this case sequential measurement using one measuring system with the round window and the basilar membrane as places of entrance was achieved.

Simultaneous measurement of the d.c. potential at the extreme end of the microcannula enabled to establish the entrance of the microcannula tip into the endolymphatic space through the basilar membrane; from the d.c. value we derived some secondary data which will be discussed later. Békésy (1952) was the first to describe the existence of the endolymphatic d.c. potential. This was later on confirmed by Tasaki et al. (1954), Gisselsson (1955), Misrahy et al. (1958), Tasaki and Spyropoulos (1959) and others.

a. Materials and technical aspects

We employed two types of pressure transducers, E. M. T. 33 and E. M. T. 35, both manufactured by the Swedish Elema–Schönander Company. The E.M.T. 33 covers a pressure range of from −30 to +30 mm Hg and has a volume displacement of 3 mm^3/100 mm Hg; its pressure chamber has an internal volume of 4 cm^3. The E.M.T. 35 – which we used only for measuring arterial blood pressure – covers a pressure range of from −300 to +300 mm Hg and has a volume displacement of 0.03 mm^3 Hg/100 mm Hg; its pressure chamber has an internal volume of 0.7 cm^3.

The principle of these pressure transducers is that any change of pressure in the pressure chamber corresponds to a change in position of the pressure membrane, and by means of a pressure rod conveys this datum to the condenser membrane. The condenser membrane is the midplate of a differential condenser; both these capacitors are parts of a bridge circuit to which an oscillator supplies a high frequency voltage. A change in the position of the midplate alters the capacities and gives rise to voltage over

the bridge due to unbalance. This voltage is then demodulated. The d.c. potential at the output side has an average value proportional to the pressure. The pressure transducers are used in conjunction with an electromanometer amplifier of the E.M.T. 31 type also produced by the Elema–Schönander Co. A description of the working of the electromanometer amplifier goes beyond the scope of this publication. The electromanometer was connected to a multichannel fluid-jet recorder (mingograf 81) also produced by Elema–Schönander which provided a possibility to record the signal.

For the endocochlear d.c. potential measurement which was performed at the same time as the pressure measurement of the endolabyrinthine fluids we used a Hewlett–Packard 412A voltmeter; its signal was amplified and also fed into the mingograf 81 apparatus.

By means of a three-way stopcock the pressure chamber of the transducer could be connected with either or both polythene cannulae (Fig. 3–1); this stopcock also allows the connection of one polythene cannula to the other. The one polythene cannula served for calibration, the other for pressure measurement. The polythene cannula primarily used for calibration we will henceforth refer to as 'calibration cannula'; the other one will be called 'measuring cannula'. The inside diameter of these cannulae was 2 mm and the wall thickness 0.5 mm.

A piece of polythene cannula (connective piece), approximately 2 cm long and having an inside diameter of 1.5 mm and a wall thickness of 0.5 mm, connected one of the 2 polythene cannulae to a pyrex microcannula (micropipette) (Fig. 3–1). The length of the measuring cannula between the connective piece and the pressure transducer was kept at a minimum (~50 cm).

The microcannula was obtained as follows: a pyrex tubing with an outside diameter of 1.8 mm and an inside diameter of 1.2 mm was provided with a conical end by means of a micro-electrode drawing apparatus. To get a microcannula with a tip suitable for our purposes we utilized a high speed rotating diamond cutting disc and an operating microscope. The microcannula was axially rotating in the procedure of cutting off the extreme end of its tip perpendicular to its axis. Care was taken to avoid cracking or pitting of the glass. We varied the outside diameter of the tips of the microcannulae from 30 to 130 microns. The corresponding inside diameters ranged from approximately 20 to 90 microns. The average length of the microcannulae was 4.5 cm. The distance between the maximal width and minimal width (tip) of the microcannula was approximately 7 mm. This provision fosters pressure communication through the microcannula. The proximal end of the microcannula which connects to the polythene connective piece was smoothed by heating it in a gas flame.

The system was filled with either normal saline solution or with Ringer's solution and for the experiments described in chapter VI occasionally with endolymph-like solution or isotonic KCl solution.

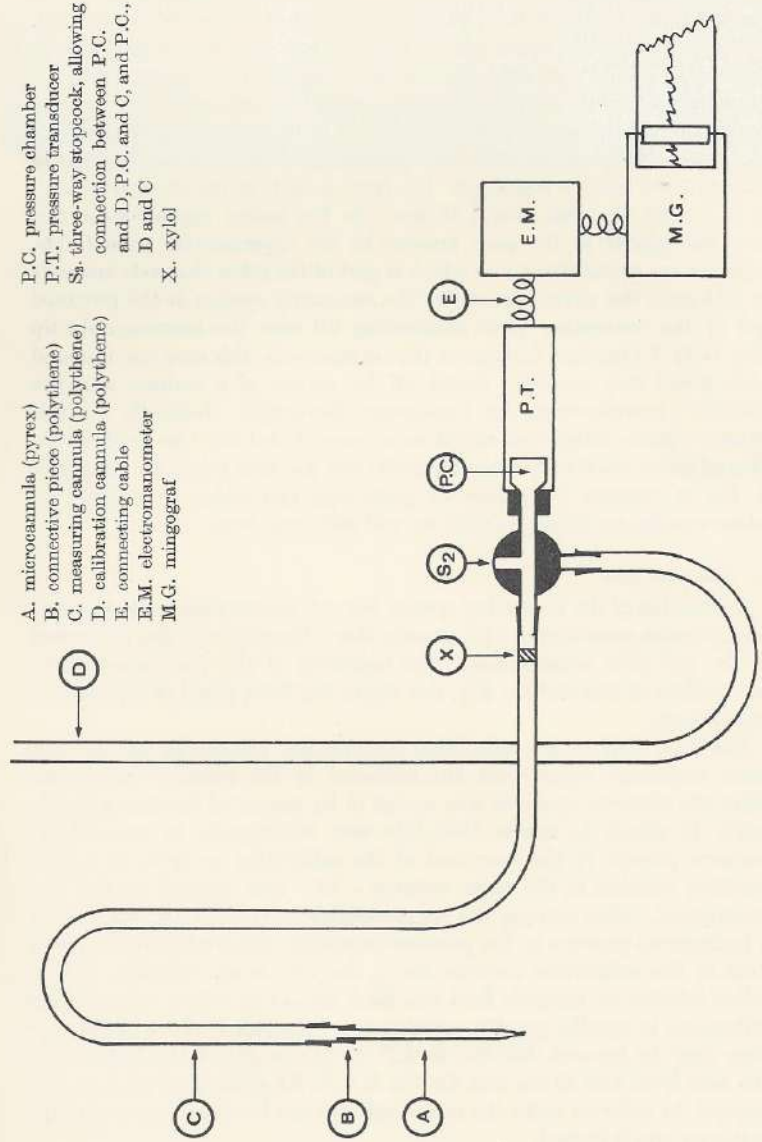

Fig. 3–1. Schematic diagram of measuring system.

At the stopcock the polythene measuring cannula was filled with xylol over a distance of approximately 0.25 cm in order to assure electrical insulation necessary for the measurement of the endocochlear d.c. potential. The xylol occupied a small volume in the horizontal part of the cannula flanked by conducting fluid (see Fig. 3–1). In our later experiments the silver wires employed in the measurement of the endocochlear d.c. potentials were chloridized in order to get rid of contact potentials. This is not absolutely essential to the experiments, because any change in potential will still be registered. The cross section of the silver wire used as a ground electrode was 1.75 mm. In the actual measurement this wire was placed in the neck muscles of the experimental animal. The cross section of the silver wire which is part of the other electrode amounts to 0.15 mm; this silver wire enters the measuring system at the proximal end of the connective piece channelling till near the microcannula tip (Fig. 3–2). To prevent leakage at the entrance site, this area was cleansed with toluol and carefully closed off by means of a contact adhesive ('Snelfix' manufactured by Cetabever, Beverwijk, Holland). To this adhesive some toluol was added in a ratio of 5:1 for this application. This adhesive was found to be very useful and has been especially important in the prevention of leakage of perilymph and endolymph along the microcannula, on which subject we will elaborate later.

b. Calibration

Calibration of the measuring system beyond the microcannula preceded each pressure measurement. Frequently this calibration was also performed at the end of a measurement. The operation of the electromanometer is described in this section. Fig. 3–3 shows the front panel of the electromanometer.

The fluid level in the calibration cannula was brought in one and the same horizontal plane with the midpoint of the pressure membrane. Then the electromanometer was zeroed in by means of the zero adjuster knob. It stands to reason that this zero corresponds to atmospheric pressure present at the open end of the calibration cannula. The most sensitive position of the range selector – 10 – was optimal for the zero adjustment. After turning the range selector button to 300 we applied a hydrostatic pressure to the pressure membrane using a known height of fluid in the calibration cannula above the zero level. Actually, normal saline solution or Ringer's fluid was used instead of water, but resulting differences in specific gravity and therefore in pressure are so slight that they may be ignored. For the E.M.T. 33 the height of the fluid above the zero level was 30 cm and for the E.M.T. 35 a height of 300 cm was applied. In order to make the meter indicate one hundred, the sensitivity equalizer was adjusted.

Electrical standardization was performed as follows: The fluid level in the calibration cannula was brought back to zero. The range selector

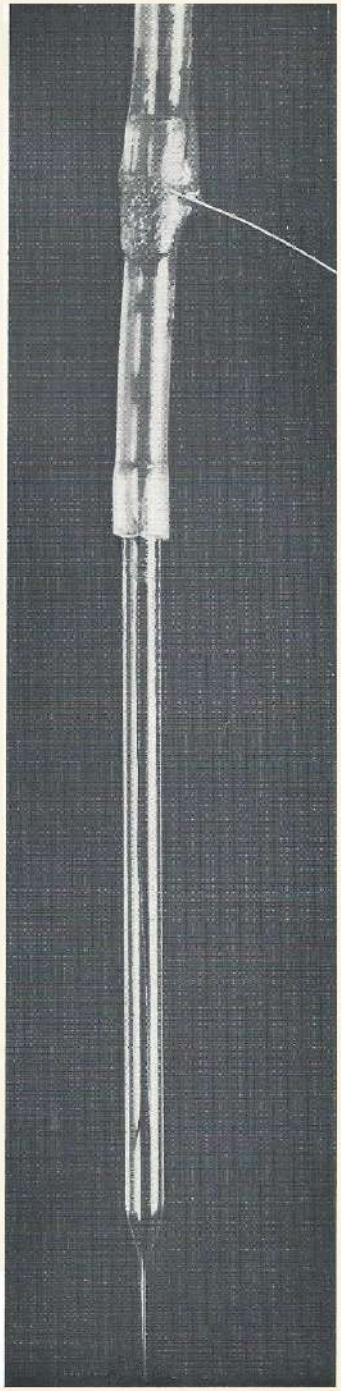

Fig. 3–2. The distal part of the measuring polythene cannula, the connective piece and the microcannula; note the silver wire entering the system between the polythene measuring cannula and the connective piece.

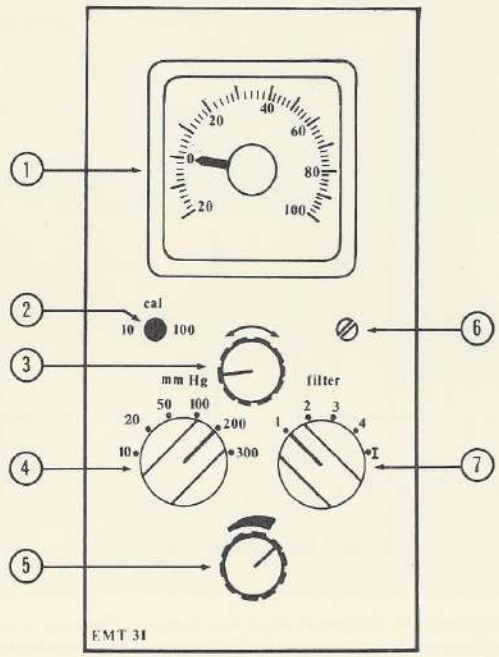

Fig. 3–3. The front panel of the E.M.T. 31 electromanometer.

1. meter
2. electrical standardization button
3. zero adjuster
4. range selector
5. sensitivity equilizer
6. standardization adjuster
7. frequency response selector

button was switched to ten or to one hundred. The electrical standardization button was pushed to produce an electrical signal. With the standardization adjuster the meter was made to indicate exactly one hundred. Electrical standardization facilitated calibration of the mingograf as it enabled to produce electrically a deflection of 100 scale divisions on the electromanometer. One hundred divisions on the meter of the electromanometer were made to correspond with a 5 cm deflection on the paper of the mingograf. It is obvious that full deflection on the meter in the positions 300, 200, 100, 50, 20 and 10 of the range selector indicates the centimeters hydrostatic pressure when the E.M.T. 35 is used and corresponds to 30, 20, 10, 5, 2 and 1 cm of water, respectively for the E.M.T. 33. Fig. 3–4 exemplifies the resulting deflection on the mingograf for the E.M.T. 33.

The linearity of the system was checked with a battery of fluid columns of various lengths and with different positions of the range selector switch. This was done repeatedly although not in every single experiment. Also

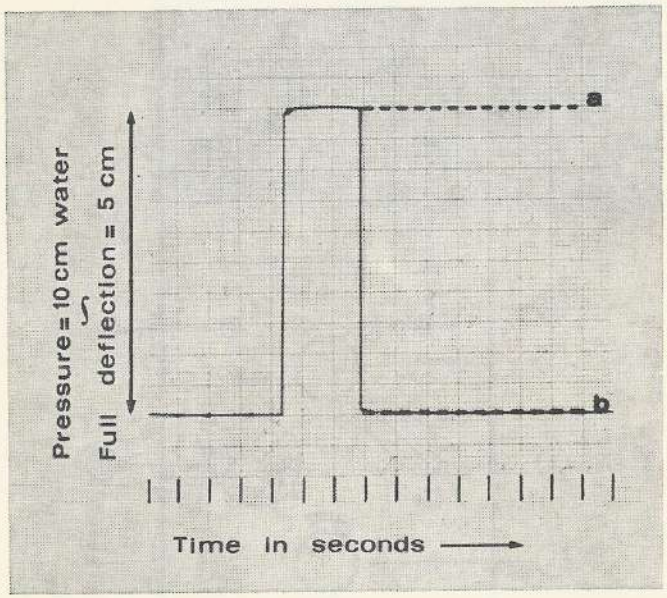

Fig. 3–4. Photograph of mingograf recording during calibration. Line b is the baseline of the recording and line a indicates full deflection of the meter in the electromanometer panel. Full deflection is reached at pressures of 30, 20, 10, 5, 2, and 1 cm of water, when the range selector knob is set at 300, 200, 100, 50, 20, and 10, respectively.

the reproducibility was checked. It should be pointed out that discrepancies between the different ranges do not affect relative measurements once a range is selected. If one is only interested in relative pressure differences, mere absolute calibration errors will be of no consequence; this eliminates an important source of errors. When we employed more than one measuring system we encountered slight deviations in pressure values between the systems, which indicates once again the drawback of using multifold systems.

On occasion we also calibrated the complete system including the microcannula (second calibration procedure) simultaneously checking the effectiveness of the contact adhesive Snelfix as a preventive against leakage. Fig. 3–5 shows the experimental set up.

To this purpose we employed a bottle of water closed by a metal cap containing two holes: one serves to apply pressure, the second one is a test hole to check leakage. The test holes of a set of these caps had diameters ranging from 150 to 1000 microns. For the sake of clarity we remark that the screw capped bottle of the second calibration procedure plays a similar role in vitro as does the endolabyrinthine space of the experimental animal in vivo, with omission of the membranes.

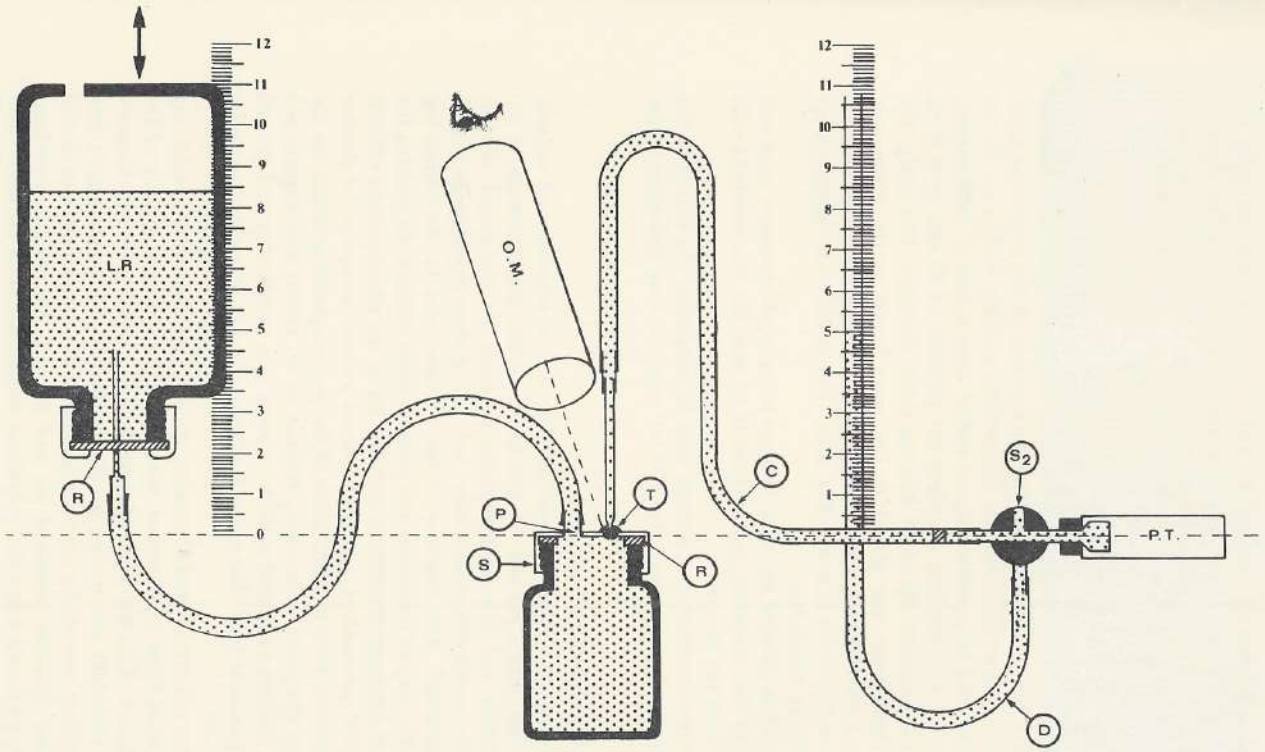

Fig. 3–5. Schematic drawing of the experimental setup to check the effectiveness of the adhesive 'Snelfix' as a preventative against leakage. This setup was also used to calibrate the complete system (second calibration procedure). Note: scales are arbitrary.

C. measuring cannula
D. calibration cannula
O.M. operating microscope
P. pressure hole
P.T. pressure transducer
R. rubber seal
S. screw cap
S_2. three-way stopcock
T. test hole, with diameters of 150–1000 microns, closed with the 'Snelfix' around microcannulae of 30–130 microns outer diameter

The pretreated tip (see chapter III under c: initial preparation) of the microcannula was placed just above the test hole in the cap of the immobilized bottle under an operating microscope and by means of a micromanipulator. When the exact entrance site – i.e. centre of the test hole – of the microcannula was thus determined, the microcannula was raised for the final preparation of its distal end. This preparation we will describe in detail when the endolabyrinthine pressure measurement will be discussed. Then the microcannula tip with an outer diameter somewhere between 30 to 130 microns was inserted into the test hole of the screw cap again using the micromanipulator. The self-settling packing Snelfix on the tip (initial and final preparation) turned out to close a hole of 1000 microns cross section withstanding a hydrostatic pressure of 30 cm, even if we used a microcannula tip of 30 microns outer diameter. Obviously, the pressure transducer was placed at the same height as the screw cap. Pressures were obtained from a water column (large reservoir) exerting pressure on the bottle of water (Fig. 3–5). Calibration of the entire system could then be concluded.

It should be pointed out that changes in hydraulic pressure were well followed by the measuring system even if we used the microcannula with smallest bore (30 microns).

The voltmeter–mingograf system used in measuring the d.c. potential was calibrated in such a way that 300 mV caused a 5 cm deflection on the paper.

c. Description of the method

As mentioned before we used the cat as the experimental animal. But, on the guinea pig also we performed several measurements of the perilymphatic and the endolymphatic pressure via the round window and basilar membrane, respectively; only one of these experiments proved to be successful. The anatomy of the relevant area in the guinea pig renders this way of measuring the pressure more difficult. In piercing the secondary tympanic membrane with the tip of the microcannula without very special precautions, leakage occurred without exception (cf chapter II). In most of their measurements of the perilymphatic pressure via the secondary tympanic membrane, Weille et al. (1958) also experienced leakage. Finding a material suitable to serve as a satisfactory seal, proved to be a laborious task. The contact adhesive Snelfix turned out to be by far the best.

Now we will give a brief discussion of the preparation of the measuring system distal from the stopcock with special reference to the tip of the microcannula. This tip was submerged in a normal saline or Ringer's solution and fluid was sucked up slowly through the microcannula into the measuring cannula by means of a syringe. This procedure was elected since from previous occasions we had learned that minute polythene particles, produced when the polythene connective piece is drawn over

the microcannula, can be brought into circulation congesting the microcannula tip if the fluid is moving downstream. Smoothing of the proximal end of the microcannula served to diminish particle formation (cf page 12). The minute particles, if any, were now sucked into the measuring cannula and syringe for removal. When the measuring cannula was totally filled with fluid, not leaving space for any air bubbles, the isolating xylol was injected with the aid of a second syringe. In doing so, fluid was forced through the tip of the microcannula which in our experience now hardly ever plugged up during this procedure. Subsequently, a small amount of normal saline or Ringer's solution was forced through the polythene cannula with a syringe so that the xylol was flanked by conducting fluid (see Fig. 3–1).

The distal end of the measuring system prepared in this way could now be attached to the fluid-filled three-way stopcock; during these manipulations extreme care was taken to prevent the formation of any air bubbles.

A description of the initial preparation of the microcannula tip will now be given. Effective use of the contact adhesive requires a clean surface. To this purpose the distal end of the microcannula was carefully, though thoroughly, rubbed with toluol under an operating microscope. Contamination of the conducting fluid with the cleansing toluol was kept at a minimum by air present at the tip of the microcannula. This air was introduced from the distal side by pinching and releasing the measuring cannula while the other side was closed off by means of the three-way stopcock (see Fig. 3–1). Subsequently, the calibration cannula and the measuring cannula were connected via the three-way stopcock. The fluid in the calibration cannula was utilized to force fluid downstream out of the microcannula thus expelling the toluol which during cleansing had been sucked in by capillary action of the microcannula tip.

The contact adhesive diluted with toluol (three parts toluol with five parts contact adhesive) was applied with the utmost care and precision in an appropriately thin layer on the distal external surface of the microcannula up to the very rim (initial preparation of the distal part of the microcannula). This operation was performed with the aid of an operating microscope. Even so the tip of the microcannula occasionally got stuck.

At this stage we calibrated the measuring system and voltmeter as outlined in the first calibration procedure we described before.

These preparations preceded anesthesia of our experimental cat with an intravenous injection of sodium pentobarbital in a dosage of 30 mg/kg body weight. A tracheotomy was performed on the cat to ensure a sufficient breathing way which otherwise could have been jeopardized by the head-holder securing immobilization. We inserted a catheter into the femoral vein in order to obtain a fast increase – if indicated – in the sodium pentobarbital level of the blood, without touching the animal. The arterial blood pressure was measured in a number of animals via a catheter in

the femoral artery; to this purpose we filled the catheter with normal saline solution to which heparin had been added, and connected the catheter to the E.M.T. 35 pressure transducer.

The posterior part of the bulla approached from the ventral side was for the larger part exposed without violating blood vessels of any importance in this area, and was opened so that the round window and the secondary tympanic membrane became clearly visible.

Cats with middle ear infection or displaying scar tissue in this region were discarded. Neither did we use a cat if an anatomical deviation rendered the animal unsatisfactory for our type of experiment.

Fig. 3–6 shows a microcannula and the basilar membrane viewed through both the tympanic membrane and the intermediate perilymph after we had opened the posterior room of the bulla. Note the few capillary vessels in the round window membrane. The cross section of the basilar membrane at the site of the round window amounts to approximately 225 microns.

The head of the cat was fixed in such a way that the membranes were in positions suitable for measurement, the tangent planes at the site of interest yielding minimal angulation with the horizontal plane.

Observed from many directions through an operating microscope, the tip of the vertically mounted, pretreated (initial preparation) microcannula was moved by means of the micromanipulator towards a point just above the secondary tympanic membrane from where a vertical path would lead to the basilar membrane where it borders the spiral ligament. The site of perforation was elected in such a way that the secondary tympanic membrane there contained no capillary vessels. To set the microcannula in the right direction requires patience combined with considerable experience.

The microcannula had to be raised 2–3 cm with the aid of the micromanipulator for the final preparation of its distal end. Blowing dry air over its surface removed condensed water from the microcannula tip. Then we proceeded with the final preparation of the microcannula. Contact adhesive and toluol were mixed in a proportion of 5 to 2. A thin iron rod was dipped into this mixture and served as a winding tool. The diluted adhesive was rather thready and could be wound around the distal end of the microcannula up to its very tip. Care had to be taken not to cover the opening of the tip. The adhesive thread generally broke during this procedure so that a cascade of applications was necessary. The resulting wound thread eventually lost its shape, flowing to a continuum around the tip end. The operating microscope was a necessary tool in this delicate operation. The reason for applying a second layer (i.e. final preparation) at this stage will now be clear since otherwise the view necessary for exact location of the microcannula would have been obstructed: the first, thin, layer is transparent (see page 19), but this lastly applied layer is opaque due to its thickness and hinders the visibility of the microcannula tip and the desired localization. Again dry air was blown over the surface

Fig. 3–6. The basilar membrane viewed through both the secondary tympanic membrane and the intermediate perilymph. A microcannula directed towards the basilar membrane is also shown.

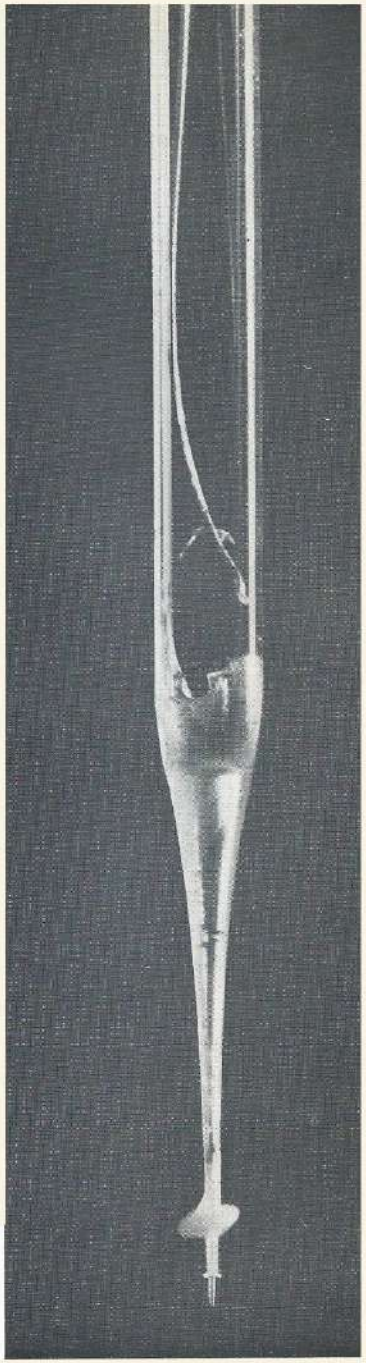

Fig. 3–7. The microcannula after retraction following a successful measurement. The upper and the lower ring of contact adhesive are the results of encounter with the round window and basilar membrane, respectively.

of the applied adhesive to obtain the desired degree of flexibility of the adhesive quickly.

We used the water column in the calibration cannula and the three-way stopcock to expel the remaining air out of the microcannula tip, which could then be lowered with the micromanipulator in order to perforate the secondary tympanic membrane for measurement of the perilymphatic pressure.

The method described above ensures a good result because the contact adhesive provides a self-settling packing, as long as the secondary tympanic membrane has not been torn excessively. Fortunately, often a certain portion of the compressible adhesive remains at the tip end of the microcannula after passing the secondary tympanic membrane, serving as the seal when the basilar membrane is pierced. This could be concluded from an inspection of the microcannula tip afterwards. Fig. 3–7 demonstrates the distal part of the microcannula after retraction following a successful measurement.

It should be mentioned that the absolute perilymphatic pressure was measured with the range switch of the electromanometer on 200, which after due time was switched to the sensitive setting of 20 for comparison of the perilymphatic and the endolymphatic pressure (see page 15). As already said in the beginning of this chapter the d.c. potential served to determine the entrance of the microcannula tip into the endolymphatic space.

d. Requisites for reliable measurements

Weille et al. (1958, 1961) and Martinez (1969) fulfilled a certain set of conditions in the performance of their experiments. In our judgement their set of conditions has not been stringent enough and we have drawn up a different and more elaborate set of requisites to which we adhered strictly. For instance, the possibility of a congested cannula had not been given sufficient consideration – as pointed out in chapter II – and had hence not been ruled out appropriately. The possibility of pitfalls in methods employed previously made us very particular in choosing our requisites, which gradually developed, along with our experience, to the final form which we will present here.

We performed many experiments before a satisfactory technique was developed for measuring endolabyrinthine pressures via the round window membrane.

If not each and everyone of the requisites was satisfied, the experiment was rejected as not representative. To start with the requisites already discussed in this and the previous chapter, these were observed and need no further digression.

Warrants to rule out leakage of perilymph along the microcannula and the secondary tympanic membrane:

1. The resulting shape of the contact adhesive after the experiment

should show a constricted belt in between two thicker rings, the proximal one of which is formed by the secondary tympanic membrane during insertion and should be sizable in comparison to the opening in the secondary tympanic membrane (see Fig. 3–7).

2. When the selector knob of the electromanometer is put on 20 (see page 15), clearly detectable variations with the frequency of respiration (see Fig. 3–9) should be present (expiration caused an increase, inspiration a decrease in the endolabyrinthine pressure); their size is influenced by the bore of the microcannula tip and the breathing depth. In many a case the heartbeat traced itself also, but its dependence upon the inside diameter of the microcannula tip was far more pronounced.

3. Careful suction of fluid from the bulla space should not change the perilymphatic pressure value.

4. Visible signs of leakage should be absent. This must be checked with the aid of the operating microscope.

The last two points alone we consider insufficient evidence for the absence of leakage.

Requisites excluding mixing of perilymph and endolymph alongside the microcannula:

1. The presence of a 'ring' shaped by the basilar membrane at the distal end of the microcannula (see Fig. 3–7).

2. Upon removal of the round window membrane – after the experiment – the basilar membrane becomes directly visible. Apart from a nearly submicroscopic spot, indicating where, at the side of the spiral ligament, the tip of the microcannula had perforated the basilar membrane, this membrane should appear normal. To visualize this spot does entail considerable effort.

3. The endocochlear d.c. potential has to keep level after perforation of the basilar membrane by the microcannula tip.

Requisites to exclude congestion of the microcannula tip when measuring the perilymphatic pressure:

1. A quick rise of the pressure to equilibrium should be seen following the perforation of the secondary tympanic membrane (see page 23); the rapidity of this rise depends to some degree on the bore of the microcannula tip also.

2. Clearly detectable pressure variations should be present and should have the same frequency as the respiration when the selector knob is put in position 20. The superimposed heartbeat variations are then present in nearly all qualifying cases; however, their presence is not considered an absolute necessity.

3. After the experiment, exertion of very slight pressure (a few mm of water) inside the microcannula tip, barely submerged in water, must result in an outflow, deduced from a descent of the water column in the calibration cannula which provided the pressure head.

Requisites to exclude congestion of the microcannula tip when measuring the endolymphatic pressure:

The last two requisites (2 and 3) for the perilymph hold for endolymphatic pressure measurements too. Whenever the tip of the microcannula meets a membrane the pressure variations due to respiration and, whenever applicable, heartbeat, disappear (see Fig. 3–11). Occasionally these variations do not reappear after the membrane is pierced. This must be interpreted as congestion of the microcannula tip, since the pressure remains at level in these cases, at least for some time, and then slowly drifts downwards. This drift could be imitated with the instruments only, omitting the experimental animal. Pressure variations related to breathing or heartbeat seldom disappear once the endolymphatic space has been entered.

General requisites:

1. The perforation hole of the basilar membrane must border the spiral ligament.
2. In going from perilymph to endolymph there should be an appropriate increase in d.c. potential (see Table III–1).
3. Of course, the general condition of the experimental animal has to be satisfactory beforehand. The animal is reevaluated during the experiment with respect to ventilation, heartbeat, only in a portion of the cases complemented by arterial blood pressure measurement; this last parameter namely proved invariably to be satisfactory in our experiments on the cat; this probably relates to the fact that the experiment lasts less than 2½ hr. and that the – minor – operation was performed with care.

e. Results

Interference with a space where pressure has to be measured will expectedly disturb the conditions present before measurement. The displacement volume (see page 11) necessary for measurement of the perilymphatic pressure exemplifies this. Fortunately the pre-puncture pressure level appears to be restored rather rapidly (the order of magnitude amounts to ¾ minute, see Fig. 3–8) and the small displacement volume is apparently produced easily (cf. chapter V, pp. 44, 45).

This conclusion was deduced from two facts.

1. Several times in a row the perilymphatic pressure built up to the same equilibrium value in repeated measurements, starting every time from atmospheric pressure which was forced upon the perilymphatic space by means of the column in the calibration cannula via the three-way stopcock. (see Fig. 3–1).
2. After the connection of a cannula with an inside diameter of 1.5 mm to the perilymphatic space, via a needle with a bore of 0.6 mm in one of our earlier experiments, we found a steady rise of fluid in this cannula until a level was reached equal to the normal perilymphatic pressure head

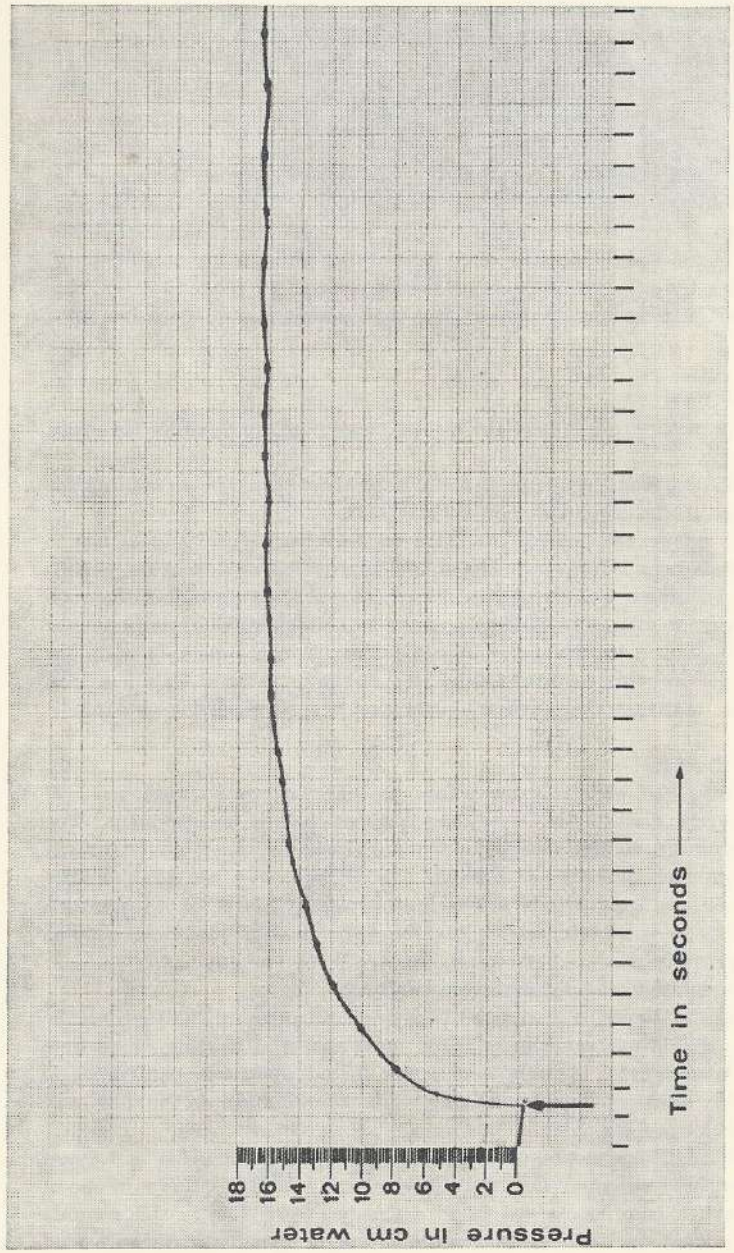

Fig. 3–8. Recording of the pressure when the microcannula tip pierces the secondary tympanic membrane at the time indicated by an arrow. Note the pressure buildup to equilibrium. The abscis gives a time scale in seconds; the ordinate a pressure scale in cm of water. The range selector switch was at position 200.

with respect to atmosphere. In this measurement the needle is introduced through a hole drilled in the cochlear wall, slightly to the medial side, about 1 mm anterior to the attachment between the secondary tympanic membrane and the round window niche. The suitability of this site was pointed out earlier by HUGHSON (1932). Leakage at the needle tip was prevented by a rubber collar. Filling the relatively large volume of needle and cannula until the equilibrium level of the perilymphatic pressure was reached required several hours; meanwhile the production slowed down gradually when the pressure in the perilymphatic compartment rose as could be deduced from the fluid level in the cannula. The initial production rate of perilymphatic fluid is in reasonable agreement with the time found necessary for the production of the displacement volume.

When the microcannula tip enters the perilymphatic space and when this tip is inserted farther, after establishing the equilibrium pressure, a very small portion of the perilymphatic space is occupied by the relevant volume of the distal part of the microcannula and the fluid in this part. This type of volume displacement expectedly results in an increase of the perilymphatic pressure. The microcannula tip was inserted in stages of 25 microns at a time, regulated with the micromanipulator. The perilymphatic pressure indeed showed the expected increments. However, they turned out to be of very short duration and were small in size; the perilymphatic pressure returns every time to the same value (see Fig. 3–9). We may conclude that the perilymphatic pressure displays a tendency towards maintaining a certain level.

Fig. 3–9 shows the perilymphatic pressure variations corresponding to the respiration with superimposed pressure variations reflecting the heartbeat. Five cm of the ordinate in this graph corresponds to a pressure of two cm of water; the range selector switch of the electromanometer was at position 20. Each time the microcannula was moved farther inwards over a distance of 25 microns, this was indicated by pressing the marking button on the mingograf 81 which produced a mark in a downward direction on the recording line. Note the shortlasting pressure increments due to the stepwise insertion of the microcannula indicated by arrows. An increment may become obscured in an upward phase of the pressure variation due to respiration.

Similar phenomena occur when the tip of the microcannula enters the endolymphatic space after piercing the basilar membrane; every time the microcannula tip penetrates 25 microns deeper, a pressure increase of short duration is noticeable.

It should be mentioned that in both the perilymphatic and endolymphatic pressure measurement the pressure line displays irregular fluctuations apart from the influences of respiration, heartbeat and insertion. At a later stage of our investigation (cf. chapter IV) corresponding irregular waves in the pressure line were also detected for the cerebrospinal fluid. The order of magnitude is a few (up to 3) mm of water in an interval of

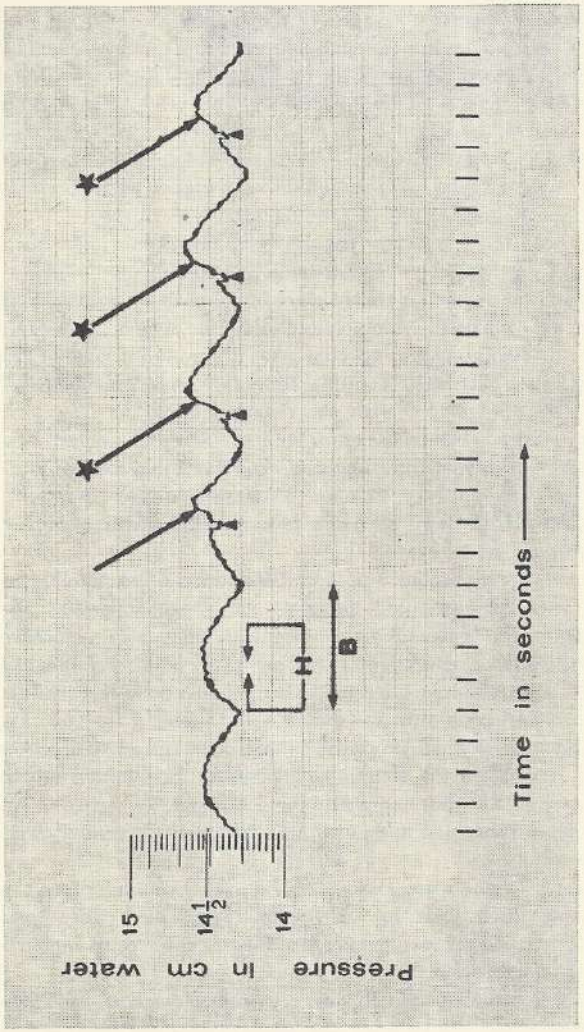

Fig. 3–9. A plot of the perilymphatic pressure versus time. The long waves are due to breathing of the cat, with breathing period B. The superimposed shorter waves reflect the heartbeat, with heartbeat period H. The sloping arrows indicate the temporary pressure increase due to displacement of perilymphatic fluid by the microcannula. This pressure increase is less noticeable in the cases marked with an asterisk. The beginning of each additional penetration over 25 microns into the endolabyrinthine space is marked in the pressure line by pressing a marking button on the mingograf. The range selector switch was at position 20.

variable duration, sometimes as short as 10 seconds. Fig. 3–10 shows an example of the variable pressure line in the measurement of the perilymphatic pressure.

FINAL RESULTS

The perilymphatic pressure proved to be equal to the endolymphatic pressure within the margin of uncertainty of the measurement (1 to 2 mm of water). Because of the endolabyrinthine pressure variations of the type referred to in Fig. 3–10, an inaccuracy of 1 to 2 mm of water in the deflected pressure gradient is to be expected if we consider the time consumed for piercing the basilar membrane (the precision of the measuring system exceeds this accuracy by far). This is the reason why the range selector knob is not switched to a position lower than 20; moreover, by refraining from position 10 the chance of going off scale is diminished. If we adhere strictly to the set of requisites mentioned earlier on, only 12 of some 80 measurements qualify as representative. These 12 experiments provided perilymphatic pressures during 1 minute before piercing the basilar membrane and endolymphatic pressures during 1 minute thereafter. We averaged these two sets of pressures, and the differences ($\triangle P$ = perilymphatic pressure minus endolymphatic pressure) are listed in Table III-1. Many other measurements confirmed the same finding of equal perilymphatic and endolymphatic pressure but did not fulfill our entire code of practice. Fig. 3–11 demonstrates the pressure variations when the microcannula tip is advanced in steps of 25 microns at a time through the perilymphatic space and the pressure variations in the endolymphatic space after the tip of the microcannula has pierced the basilar membrane. These measurements were carried out with the range selector knob at position 20, five cm deflection on the ordinate corresponding to two cm of hydraulic pressure. Note the interesting phenomenon that the basilar membrane when touched by the microcannula, first removes the pressure variations due to heartbeat and thereafter those due to respiration. These variations register again after the microcannula tip has pierced the basilar membrane. Pressure variations reflecting the respiration and heartbeat are equally demonstrable in the endolymphatic pressure curve and match the perilymphatic curve of the same animal.

In particular our aim was to measure the difference, if any, between the perilymphatic and endolymphatic pressure; to this purpose the range selector knob was switched to the more sensitive position 20 from the original position 200, after compensating the absolute deflection with the zero adjuster. A pressure difference of 2 cm of water then caused a deflection of 5 cm where it previously would have made the writing pen deflect 0.5 cm (see page 15). Knowledge of the absolute pressure value is not lost by changing the position of the range selector knob: The absolute pressure value is known from a reading with the range selector knob at position 200; the position of the writing pen after the new setting

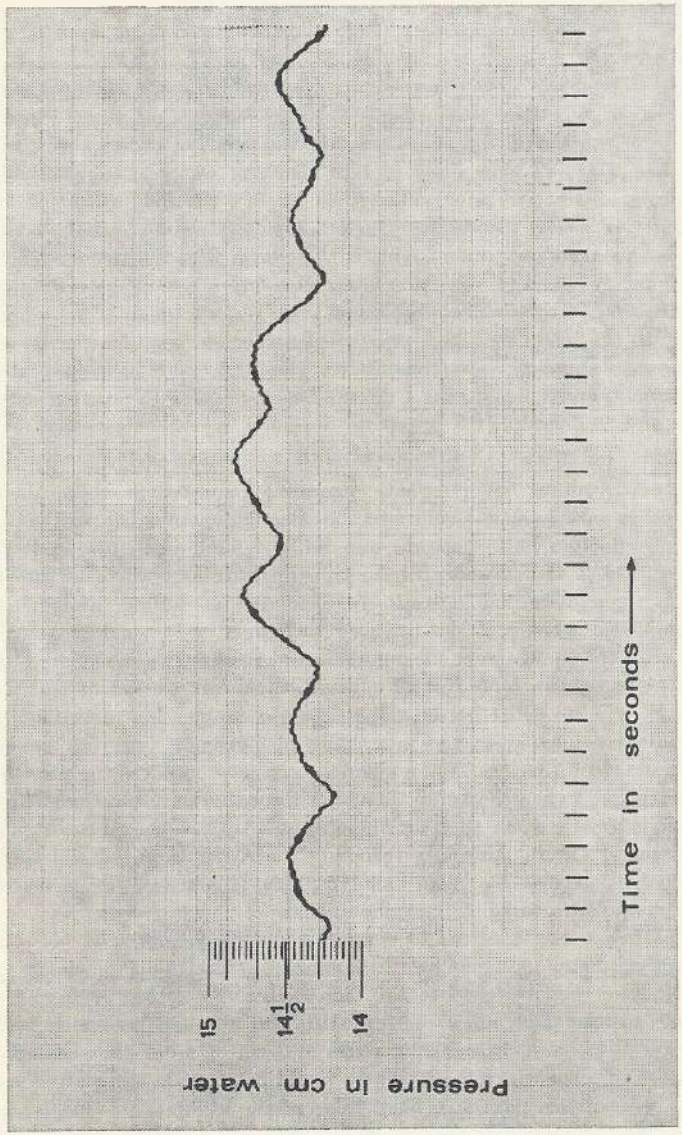

Fig. 3-10. A fluctuation in the perilymphatic pressure line with superimposed influences of respiration and heartbeat.

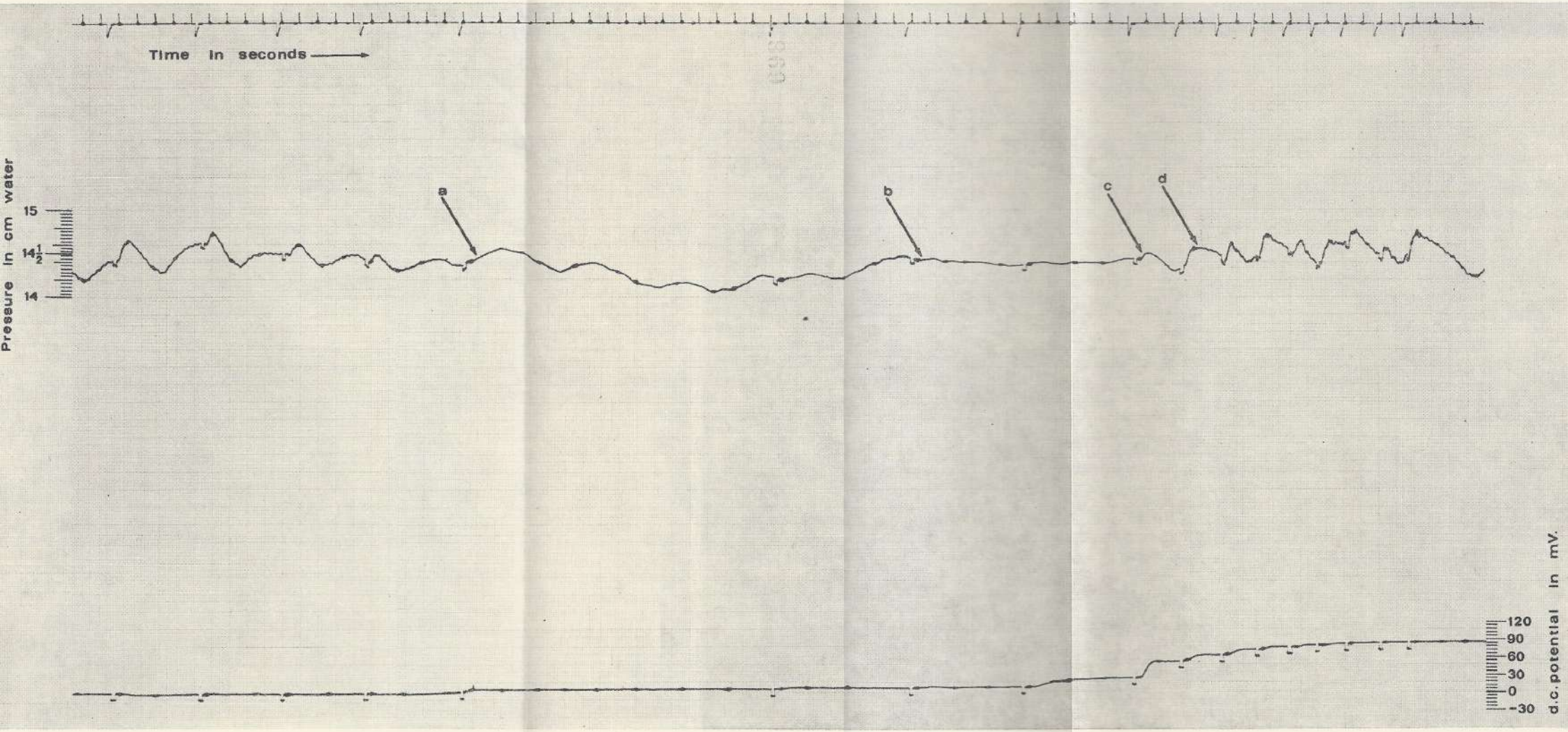

Fig. 3–11. A plot of pressure and potential versus time. The middle line indicates the pressure (as read on the left ordinate) in advancing the microcannula tip by 25 micron increments at a time: on the left in the perilymphatic compartment, on the right in the endolymphatic compartment; in the intermediate region the microcannula touches and pierces the basilar membrane.

a. end of heartbeat fluctuation
b. end of respiration fluctuation
c. reoccurrence of respiration fluctuation
d. reoccurrence of heartbeat fluctuation

The bottom line indicates the d.c. potential as read on the ordinate on the right. The marks in downward direction, shown on all registration lines, indicate the beginning of each additional penetration over 25 microns into the endolabyrinthine space and are artificial. The topline functions as a time indicator.

of the range selector knob and of the zero adjuster still corresponds to this same absolute pressure value.

Table III-1: Absolute pressure values for perilymph, differences in pressure between perilymph and endolymph, and values for the endocochlear d.c. potential.

Experimental cat number	Perilymphatic pressure in cm water	ΔP in mm of water (see text)	Endolymphatic d.c. potential in mV
39[a]	11.4	0.8	96
37[a]	12.6	0.0	90
35[a]	13.0	1.2	84
31[a]	13.6	0.0	87
33[a]	13.8	−0.4	96
23	14.2	−2.0	90
42[a]	14.4	0.0	87
17	14.8	0.0	108
25	14.8	2.0	72
19	15.2	−0.4	102
41[a]	17.2	1.0	84
34[a]	18.0	−1.2	96

The spread in the absolute pressure values would have been less if the vertical distance from the relevant cochlea to the spine of the animal had been constant in all 12 cases; the position of the animal's head relative to its trunk influences the endolabyrinthine pressure. This will be elaborated on in chapter IV. The animal's head was positioned in such a way that the membranes were in a position suitable for measurement. As a consequence, small anatomical variations of the round window and the secondary tympanic membrane influenced the position of the head and hence caused differences in the vertical distance between the cochlea and spine for different animals.

The one successful measurement on the guinea pig (which we mentioned before) also yielded equal pressure values for perilymph and endolymph, within the limits of accuracy of our experiment. Only a subset of the requisites as previously listed was observed in this case, but we still like to consider this measurement as qualifying. The blood pressure was not measured; the superficial breathing hardly reflected on the rather fluctuating pressure line; the heartbeat influences on the other hand, showed clearly; the absolute perilymphatic (or endolymphatic) pressure was 7 cm of water and the endocochlear potential 78 mV.

As mentioned before, we used the endocochlear d.c. potential as a localization parameter. As shown in Fig. 3–11 this potential increases by some 90 mV when the endolymphatic space is being entered. Piercing of the basilar membrane with the microcannula tip just before the endolymphatic space is entered frequently produces a negative dip in the d.c. potential before the eventual rise towards the endolymphatic level

(Fig. 3–12). This is in agreement with findings of VON BÉKÉSY (1952) and is caused by a different d.c. potential inside the cells of the basilar membrane. The values we found for the endolymphatic d.c. potential are listed in the last column of Table III-1.

The technique we have developed can also be applied to the measurement of the 'distance'[1]) from the basilar membrane to Reissner's membrane, since from the micromanipulator readings the displacement of the microcannula tip can be determined quantitatively, while the following two phenomena exist as independent indicators of the location of the tip:

Firstly, the pressure variations due to respiration disappear when the microcannula tip is closed off by the basilar membrane and recover once the endolymphatic space has been entered and disappear again when Reissner's membrane is reached.

Secondly, the pressure variations reflecting the heartbeat vanish when the basilar and Reissner's membrane close off the microcannula tip respectively, recovering in between when the microcannula tip is inside the endolymphatic space.

We believe this to be a first step towards an in vivo determination of the location of Reissner's membrane relative to the basilar membrane. Possibly endolabyrinthine hydrops could thus be detected. After obliteration of the endolymphatic sac in the guinea pig, KIMURA and SCHUKNECHT (1965) and KIMURA (1967) concluded from subsequent histological examination that hydrops thus could be produced. It should be possible to check this conclusion also by the technique we advance for measuring the distance between the membranes.

Distance measurement performed on 5 cats yielded values ranging from 475 to 600 microns, with a mean value of 500. For more precise distance measurements it is desirable to ensure a direction of the long axis of the microcannula relative to the basilar membrane more constant than we have employed. Obviously, the direction in which the distance is measured can make a considerable difference. In measuring the distance positioning is important. If the head of each experimental animal is always fixed in the same way, without rendering the position of the membrane unsuitable for pressure measurement[2]), the direction of the microcannula relative to the basilar membrane may be more constant. Such will probably be the case if the experimental animals are chosen from a genetically pure strain.

To determine whether Reissner's membrane bulges in a manner as histological findings in cases of Ménière's disease seem to indicate the microcannula tip should preferably touch Reissner's membrane somewhere

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[1]) 'distance' measured in a direction nearly perpendicular to the basilar membrane at the side of the spiral ligament, henceforth referred to as distance unless specified otherwise.

[2]) Pressure measurement in this case is not the purpose in itself, but only a means to an end.

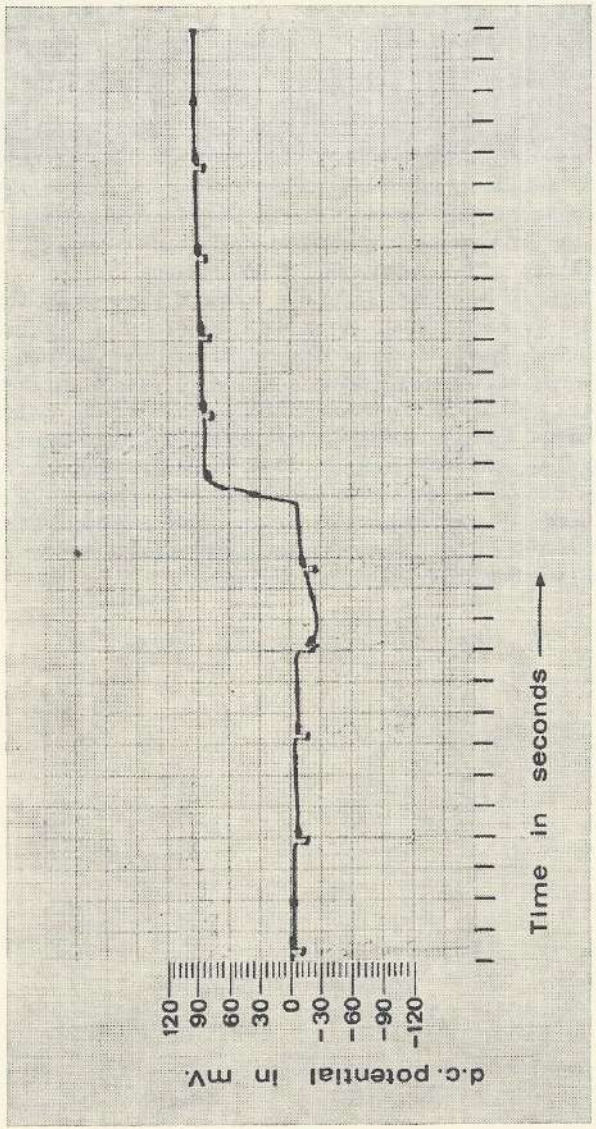

Fig. 3-12. The changing d.c. potential along the path traversed by the tip of the microcannula. The potential in mV is read on the ordinate. Note the negative dip ascribed to piercing of the basilar membrane at the side of the spiral ligament and the subsequent rise to 96 mV. The small marks in downward direction indicate an increase in penetration over 25 microns into the endolabyrinthine space.

along its midline without violating the obvious requirement that pressure measurement is still possible. We like to mention that distance measurement was not our specific aim.

Using the rise and fall in the d.c. potential as the indicator for the entrance and exit spots in the endolymphatic space is not very reliable in defining the location of these spots, because the upward and downward change of the d.c. potential in meeting the basilar membrane and Reissner's membrane respectively, is more gradual and not steep whilst encounter with Reissner's membrane presumably causes bending – and on occasion tearing – thus postponing the downward change. Distance measurements in 12 cats using the d.c. potential as parameter yielded values varying from 400 to 850 microns, with a mean value of 750 microns.

Measuring distance by means of the d.c. potential has recently been undertaken also by PEAKE et al. (1969). With the axis of a measuring microcannula oriented perpendicularly to the basilar membrane they moved the tip of this cannula towards Reissner's membrane passing the organ of Corti and the scala media. They found higher values for the distance between basilar and Reissner's membrane (~700 microns) than measured on a histological section (~350 microns). These authors mention bending of their rather thin (a few microns to a few tenths of a micron) cannula as a possible source of error. Because of the size of the microcannula tip we employed, only the second source of error appears important for our measurements, viz. bending of Reissner's membrane.

CHAPTER IV

THE RELATION BETWEEN THE PRESSURE OF THE ENDOLYMPH, THE PERILYMPH, AND THE CEREBROSPINAL FLUID

In this chapter as well as in the next one the cochlear aqueduct plays an important role. A consultation of the literature dealing with the cochlear aqueduct seems therefore indicated. The cochlear aqueduct is divided into two parts:

1. a bony canal – the canaliculus cochleae –,
2. its contents, the connective tissue inside – the ductus perilymphaticus – which consists of the dura mater and the arachnoidea (Nomina Anatomica 1966).

Conventionally, the otological authors speak of the cochlear aqueduct without further specification. We will conform to this practice.

The discovery of the cochlear aqueduct goes back to DU VERNEY (1684), who did not yet distinguish between the cochlear aqueduct and the canal of the inferior cochlear vein. COTUGNO (1774) was the first to make this distinction and to describe the bony canal covered by the dura mater, going from a niche at the round window towards the cranial cavity.

A great many investigators have tried to find an answer to the question whether and to what extent the cochlear aqueduct is patent. This answer is involved in questions as: Where does the perilymph originate?; can infections spread via the cochlear aqueduct and are the cerebrospinal and perilymphatic fluid pressures in balance? These questions must have brought about the large interest in the question of patency or non-patency of the cochlear aqueduct.

Using histological techniques, quite a few investigators studied the patency of the cochlear aqueduct. To our knowledge MEURMAN (1930) was the only one among these experimenters who described a bony obliteration (over a short distance) of the cochlear aqueduct in 4 out of 55 human temporal bones which he investigated. We wonder whether the sections in these 4 cases did not miss the cochlear aqueduct. So did PALVA and DAMMERT (1969), who themselves performed extensive histological studies on the human cochlear aqueduct. In reference to Meurman's text they suggested: 'that there was total obliteration in none and that, possibly, the sections did not represent the area of the aqueduct.'

Whether or not the cochlear aqueduct is completely obliterated by soft tissue does not only depend upon the species under investigation, but also on the 'species of investigators'. Compare for instance the results of MEURMAN (1930) with those of PALVA and DAMMERT (1969). In our

judgement the results of Palva and Dammert are the more reliable ones since it is not unlikely that the observation of a complete soft-tissue obliteration is based on artefacts. It is commonly accepted that in our experimental animal, the cat, no soft-structure occlusion exists (WINCKLER 1963).

The length and cross-section of the aqueduct were measured by several investigators (KARLEFORS 1924, MEURMAN 1930, LEMPERT et al. 1952, WERNER 1960, ANSON 1964, ANSON et al. 1965, RITTER and LAWRENCE 1965, NEIGER 1968 and PALVA and DAMMERT 1969) in various animals and in man. The results were very inconsistent.

WALTNER (1948) described the barrier membrane – membrana limitans – to be situated in the internal opening of the aqueduct. He concluded that under physiological conditions diffusion, but no direct flow, occurred between the cerebrospinal fluid and the perilymph through the barrier membrane. He found such a membrane, two or three cell-layers thick, in human fetuses. In adult human beings he described the membrana limitans as a one cell-layer membrane, one micron or less in thickness. In a later investigation ALTMANN and WALTNER (1947) could not demonstrate the presence of a continuous membrane in all consecutive sections. A membrana limitans was also described by NEIGER (1968) in monkeys. PALVA and DAMMERT (1969), who found a barrier membrane at the orifice of the cochlear aqueduct in the scala tympani in 2 out of 20 human temporal bones, concluded that as a rule, the fluid exchange between the cerebrospinal space and the scala tympani is not obstructed by any membrane.

Besides the previously discussed histological investigations, many physiological experiments have been carried out with dyes in order to confirm or to rule out the permeability of the cochlear aqueduct. Most experimenters injected the dye suboccipitally (CHILOW 1923, MEURMAN 1930, ALTMANN and WALTNER 1947, GISSELSSON 1949, GRAF and PORETTI 1950, SCEVOLA et al. 1950, ALTMANN and WALTNER 1950a, 1950b, SVANE–KNUDSEN 1958) and investigated whether it could be recovered from the scala tympani. Some investigators studied the route in the opposite direction (YOUNG 1949, ALTMANN and WALTNER 1950a, 1950b) and tried to recover the dye in the subarachnoidal space. The majority of the experiments showed passage of the dye.

Several studies have been performed on the free passage of corpuscles through the cochlear aqueduct (KARBOWSKI 1921, 1930, NYLEN 1923, MEURMAN 1930, JAMPOLSKY 1935, 1963, ALTMANN and WALTNER 1947, ARNVIG 1951, LEMPERT et al. 1952, SCHREINER 1961, 1963 and SCHUKNECHT and EL SEIFY 1963). In the majority of cases this method also revealed the permeability of the cochlear aqueduct. In this context the experiment of SCHUKNECHT and EL SEIFY (1963), who suboccipitally injected chicken erythrocytes into the cerebrospinal space of cats (both with and without previous surgical closure of the cochlear aqueduct), deserves special attention. Without surgical closure erythrocytes were recovered in the

scalae tympani and vestibuli; with a closed cochlear aqueduct no trace of erythrocytes was found in the perilymphatic space.

Some experiments with radioisotopes (SCHREINER 1961, 1963 and 1966) suggest the existence of an open connection between the perilymphatic and the cerebrospinal fluid, but in others (PORTMANN et al. 1954) no patency is found.

While on the one hand the proof of an open connection between perilymph and cerebrospinal fluid suggests a pressure equilibrium between these fluids, the experiments in which pressure measurements are performed throw some light on a possible free passage. Although CHILOW (1923), MEURMAN (1930) and SZASZ (1927) still lived under the impression that considerable pressure was required to force fluid through the cochlear aqueduct, HUGHSON (1932) concluded from his experiments in the cat that the change of the cerebrospinal fluid pressure after intravenous injection of a hypotonic or hypertonic salt solution is immediately followed by a rise or fall respectively in the intralabyrinthine pressure; he mentions that the peak in the labyrinthine pressure lags behind the extreme of the cerebrospinal fluid pressure. KOBRAK (1933, 1934) showed that pressure variations of sufficient size in the intracranial space could also be detected in the labyrinth, provided the frequency was not too high. The experiments of AHLEN (1947) and KREJCI and BORNSCHEIN (1951) also point into the direction of a pressure balance between cerebrospinal fluid and perilymph. KERTH and ALLEN (1963) found in their experiments in cats that an increase of cerebrospinal fluid pressure was more or less accurately followed by the perilymph pressure, whilst obliteration of the cochlear aqueduct completely prevented this response. Finally, MARTINEZ (1969) investigated the perilymphatic and the cerebrospinal fluid pressure in cats and guinea pigs. For the mean absolute value of the perilymphatic pressure in the cat he found 4.42 mm Hg and for the cerebrospinal fluid 5.39 mm Hg. He found a similar pressure difference of approximately 1 mm Hg in the guinea pig. He concluded that direct communication between these fluids would appear unlikely. In contrast to this, Martinez also derives from his experiments that when the cochlear aqueduct was patent, injection into the subdural space of a physiological sodium chloride solution produced an immediate increase of pressure inside the perilymphatic fluid space, while no change occurred when the aqueduct was obstructed.

It is the general consensus that in the cat the cochlear aqueduct allows direct communication of the cerebrospinal fluid and the perilymph.

OUR EXPERIMENTS

The final goal of this part of our research was to study the influence of an increase in the cerebrospinal fluid pressure on the pressures of the perilymph and, especially, of the endolymph. The influence of an increase in the cerebrospinal fluid pressure on the perilymphatic pressure was investigated by KERTH and ALLEN (1963), and others.

To our knowledge, MARTINEZ (1969) was the only one who did not only investigate the influence of an increase in the cerebrospinal fluid pressure on the perilymphatic fluid, but also on the endolymphatic fluid; he used guinea pigs for these experiments. By intravenous injection of more than half a ml isotonic saline solution, he produced a transient rise in cerebrospinal and perilymphatic fluid pressure, while the endolymphatic pressure was not affected. He also studied the influence of an anaphylactic shock on the pressure of the cerebrospinal fluid, the perilymph, and the endolymph and found a different influence on each of them. Also in this respect it will appear that our results disagree with those of Martinez.

In addition to what we set out to study in this chapter, – namely the influence of a change in the cerebrospinal fluid pressure on the pressures of the perilymph and endolymph – we also hit on some aspects which we consider an interesting by product that will be briefly discussed later on.

METHODS AND MATERIAL

The experimental cat was anesthetized with pentobarbital sodium and a tracheotomy was performed. Lumbar laminectomy served to expose the dural sac. We employed an operating microscope and made a hole in the dura with a fine needle. An intravenous catheter (intracath No. 1619 K, manufactured by C.R. Bard inc., Mary Hill, N.Y., U.S.A.) was provided with side holes at the distal end and inserted, via the opening in the dura, into the cerebrospinal space. Whenever this manipulation caused a bleeding the experiment was terminated, as this bleeding could influence the cerebrospinal fluid pressure. Leakage along the catheter was prevented at this stage by applying Eastman 910[1]) adhesive all around the site of insertion. Further fixation of the catheter was assured by filling the wound around the catheter with miniature pieces of gauze, after which the external wound was closed. The catheter was connected to a pressure transducer E.M.T. 33 which was carefully calibrated (see chapter III for details).

Subsequently all actions necessary for measuring the intralabyrinthine pressure were executed as previously described (see chapter III). For measurement of the cerebrospinal fluid pressure the pressure transducer was placed at the same height as the one used for intralabyrinthine pressure measurement (cf Fig. 4–1).

After the initial pressure measurements of cerebrospinal fluid and perilymph, we induced an increase in the pressure of the former fluid. This was accomplished by turning the three-way stopcock (stopcock S_1 of Fig. 4–1) allowing the application of an additional pressure by means of a normal saline column via the calibration cannula. Fluid then flowed into the cerebrospinal fluid compartment; because the cannula is connected to a large reservoir a nearly constant level of the fluid column is assured.

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[1]) Manufacturer Kodak Company U.S.A.

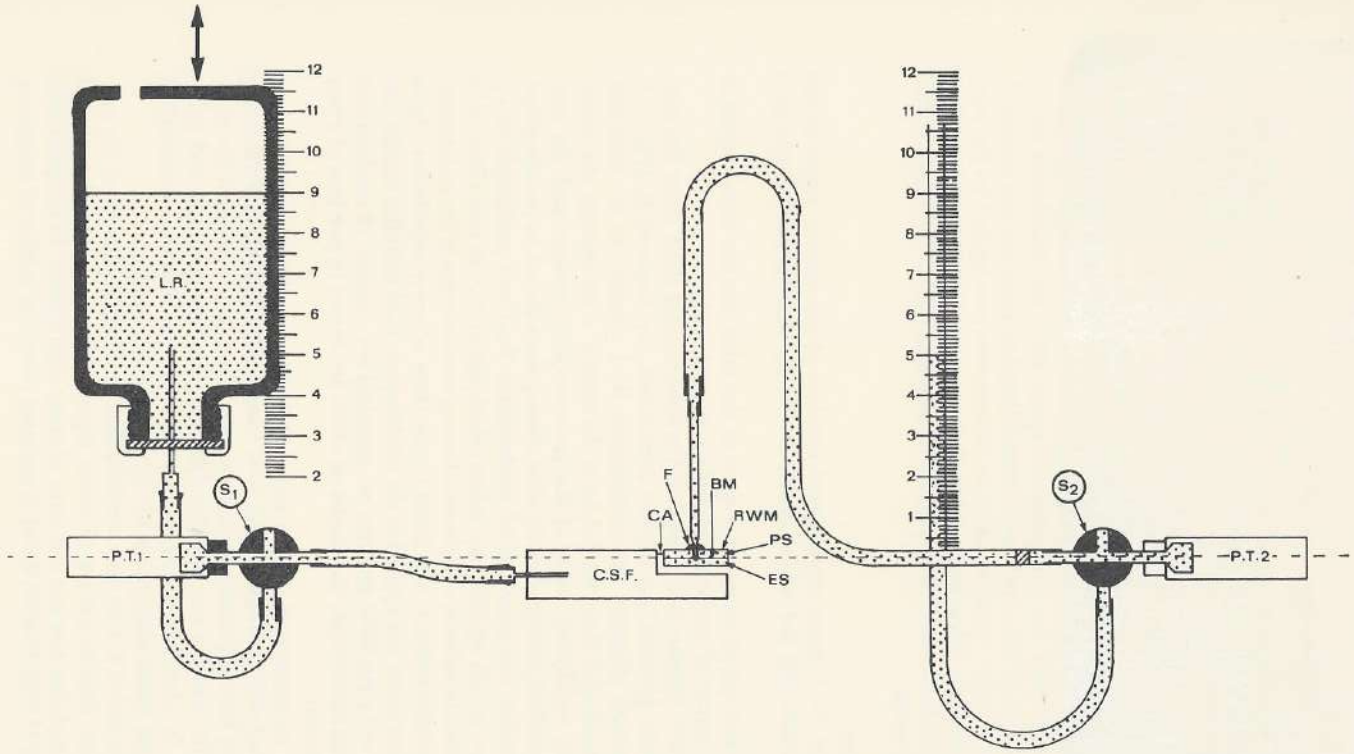

Fig. 4–1. Schematic drawing of the in vivo experiment for perilymphatic, endolymphatic and cerebrospinal fluid pressure measurements and of the provision to apply a pressure head to the cerebrospinal fluid.

B.M. basilar membrane
C.A. cochlear aqueduct
C.S.F. cerebrospinal fluid
E.S. endolymphatic space
F. contact adhesive
L.R. 'large reservoir'
P.S. perilymphatic space
P.T.$_1$, P.T.$_2$. } pressure transducers E.M.T. 33
R.W.M. round window membrane
S_1, S_2. stopcocks

Meanwhile we continued measurement of the perilymphatic pressure (cf right hand side of Fig. 4–1); after about four minutes this pressure has reached its equilibrium value. The three-way stopcock (S_1) was then turned to cut off the fluid column, leaving a connection between the cerebrospinal fluid and the pressure transducer. Subsequently, both the cerebrospinal fluid pressure and the perilymph pressure were measured and compared with each other. When these pressures had stabilized to constant values, we brought the microcannula tip through the basilar membrane into the endolymphatic space using the micromanipulator. At this stage of the experiment the endolymphatic pressure was measured. By turning the stopcock, again an overpressure was applied to the cerebrospinal fluid. When the endolymphatic pressure seemed to have reached its equilibrium value this pressure was compared to the cerebrospinal fluid pressure.

Results

Table IV–1 shows experimental numbers at the various stages of the experiment. For the sake of clarity we shortly describe these stages once again.

The first row of every experiment in this table lists the initial values of the pressures of cerebrospinal fluid and perilymph.

A water column was applied to the cerebrospinal fluid. After the perilymphatic pressure had reached equilibrium, the stopcock S_1 (Fig. 4–1) was turned, allowing the cerebrospinal fluid pressure to be measured. The second row shows the height of the applied water column, the corresponding equilibrium value of the perilymphatic pressure, and the cerebrospinal fluid pressure directly after turning the stopcock as just described.

Hereafter, the pressures of cerebrospinal fluid and perilymph slowly drop to certain equilibrium values which are shown in row three. Now the basilar membrane was pierced allowing measurement of the endolymphatic pressure which is also listed in row three of every experiment. Again an overpressure was applied to the cerebrospinal fluid and after equilibrium of the endolymphatic pressure we measured the pressure of cerebrospinal fluid and endolymph, the results of which are shown in row four of every experiment.

The measurements of the endolabyrinthine pressures and the cerebrospinal fluid pressure (cf Fig. 4–1) were performed with two measuring systems, having pressure transducers and electromanometers of the same type. The error in any pressure difference between perilymph and endolymph is, therefore, much smaller than the one between cerebrospinal fluid and either endolymphatic or perilymphatic pressure.

From table IV–1 we may conclude that the average pressure of the cerebrospinal fluid is 0.8 cm of water higher than that of perilymph and endolymph (see row one and three of every experiment). As pointed out in chapter II there is much doubt in our mind about the validity of comparing

results obtained with two measuring systems in case a large degree of accuracy is required. The pressure difference measured in this way may not be real. However, we feel obliged to report on three earlier experiments in which we used only one pressure transducer, while a three-way stopcock enabled us to alternate between the cerebrospinal fluid and the perilymphatic one. In these experiments also, the cerebrospinal fluid pressure exceeded that of the perilymph by a small margin of about 0.6 cm of water. However, the physiology of the experimental animal was definitely more disturbed in these supplementary experiments. We drilled a hole in the bony cochlear wall at the site suggested by HUGHSON (1932) and immobilized

Table IV-1

Pressures of cerebrospinal fluid, perilymph, and endolymph at equilibrium; overpressures were applied to the cerebrospinal fluid by means of a water column (large reservoir), for experimental setup confer to Fig. 4–1.

Identification number of the cat	Height of water column (cm of water) applied to cerebrospinal fluid	Pressure of cerebrospinal fluid (cm of water)	Pressure of perilymph (cm of water)	Pressure of endolymph (cm of water)
13	[1]	11.4	11.2	—
	16.2	15.9	15.6	—
	[2]	14.8	14.6	14.6
	19.4	18.6	—	18.5
18	[1]	21.6	20.2	—
	25.8	25.2	23.9	—
	[2]	21.6	20.3	20.4
	26.4	25.8	—	24.6
22	[1]	16.5	14.8	—
	21.9	20.8	19.4	—
	[2]	16.8	15.8	15.9
	30.4	28.8	—	27.9
26	[1]	18.6	18.0	—
	24.0	23.1	22.5	—
	[2]	18.9	18.3	18.3
	25.2	24.0	—	23.4
29	[1]	13.2	12.9	—
	18.1	17.1	16.6	—
	[2]	13.9	13.4	13.4
	20.2	19.0	—	18.4

[1] no external pressure on the cerebrospinal fluid.

[2] stopcock (S_1 see Fig. 4–1) blocked the connection between the pressure column (large reservoir) and the cerebrospinal fluid; during this blockage the microcannula tip pierced through the basilar membrane after which the endolymphatic pressure could be measured.

a metal cannula with a large amount of Woods metal and some Eastman 910 adhesive. We, therefore, feel less confident about the reliability of the results of these three experiments.

As mentioned already, MARTINEZ (1969) reported an average pressure difference both in the guinea pig and the cat of 1 mm Hg. This finding would be in agreement with ours, although in our opinion the reliability of these findings is questionable. In itself, a possible explanation of this pressure difference might be a continuous flow from the cerebrospinal space through the cochlear aqueduct into the perilymphatic space, where constant resorption would occur. This is only conjecture and definitely no fact, but the explanation it offers is intriguing. It would be interesting to answer the question of existence and size of this pressure difference conclusively.

The increase of the cerebrospinal fluid pressure due to a water column

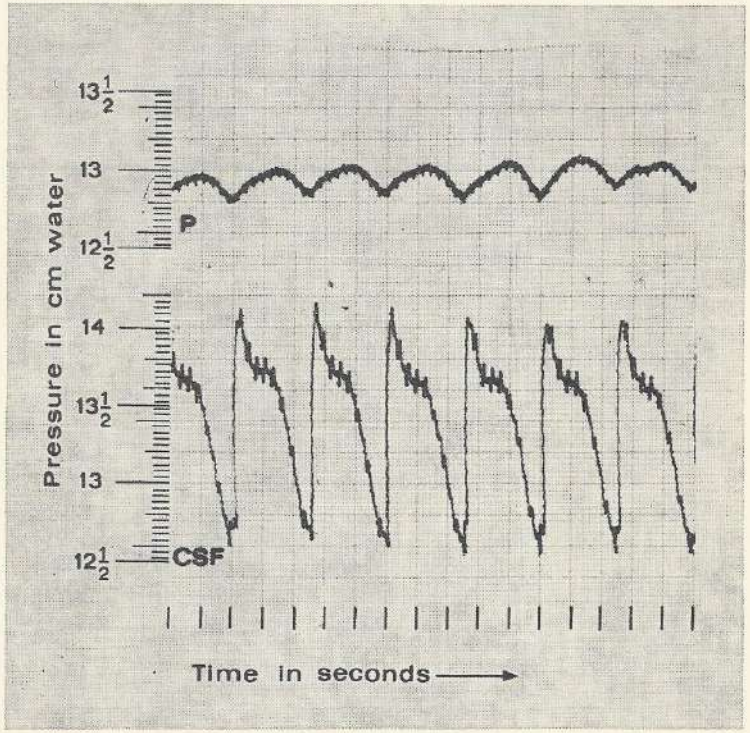

Fig. 4–2. Plot of pressure variations in the cerebrospinal fluid (CSF) due to breathing and the reflection thereof in the perilymphatic pressure (P). Note the attenuation occurring in the upper curve. A careful inspection also reveals that this curve lags behind the cerebrospinal fluid curve.

applied to this fluid is – within the small errors of our measurements – equal to the increase of the perilymphatic and also the endolymphatic pressure. Table IV–1 reveals this, as can be seen if for each experiment one compares the data presented in row one to those in row two or the data in row three to those in row four.

A few final findings with respect to the cochlear aqueduct will conclude this chapter: Fig. 4–2 shows that the variations in pressure of the cerebrospinal fluid due to breathing are reflected in the perilymphatic pressure, but that a good deal of attenuation occurs. In order to check how much of this attenuation might have been caused by the pyrex microcannula we connected this small tipped cannula (80 microns) to the much wider polythene cannula through which previously the cerebrospinal fluid pressure variations had been measured. The microcannula indeed had a definite smoothing effect on the curve as is shown in Fig. 4–3. (A comparison of the measurements presented in Figs. 4–2 and 4–3 reveals that there is a time lag between the perilymphatic pressure and the cerebrospinal fluid pressure fluctuations. A similar result was obtained for the endolymph.) The cochlear aqueduct appears to have an even stronger smoothing effect which is understandable if we consider its small effective cross section. Possible volume changes of the endolabyrinthine space due to a flexible round window membrane may contribute to this smoothing effect. The following phenomenon relates to this: Application of abdominal pressure

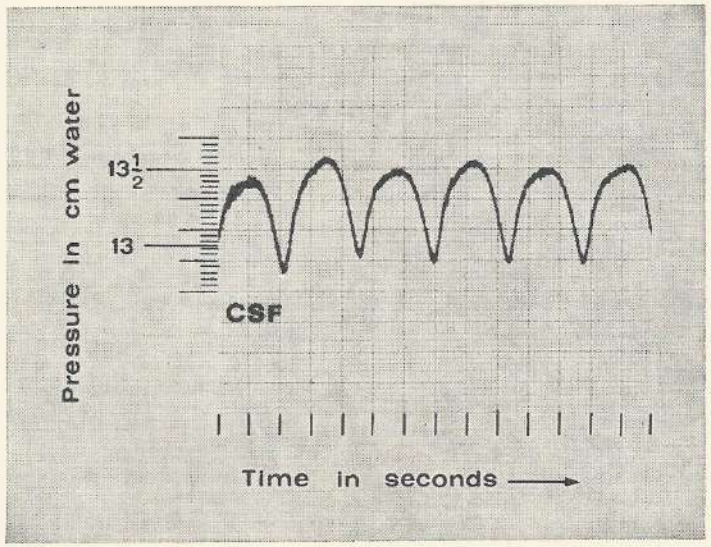

Fig. 4–3. A demonstration of the smoothing effect on the pressure fluctuations in the cerebrospinal fluid (CSF) by a microcannula with a tip of 80 microns outer diameter.

increases the cerebrospinal fluid pressure by a factor of up to two or three, which is coupled with an equal rise in perilymphatic pressure. However, the perilymph pressure reaches its top slowly upon an abrupt rise in cerebrospinal fluid pressure; when abdominal pressure is not applied any longer, the abrupt fall in cerebrospinal fluid pressure is accompanied by a slower but equal fall in perilymphatic pressure. The above is equally applicable to perilymph and endolymph. The presence of heartbeat fluctuations in Fig. 4–2 and the absence thereof in Fig. 4–3 suggest that heartbeat fluctuations in the endolabyrinthine fluid pressure occur independently of those in the cerebrospinal fluid.

Chapter V

THE INFLUENCE OF OBSTRUCTION OF THE COCHLEAR AQUEDUCT ON THE PERILYMPHATIC AND THE ENDOLYMPHATIC PRESSURE

In the previous chapter we concluded that pressure increases in the cerebrospinal fluid are followed by the same pressure increases in perilymph as well as in endolymph. Keeth and Allen (1963) and Martinez (1969) showed absence of any such response in the perilymph when the cochlear aqueduct was obstructed.

In 1933 Uyama reported a displacement of Reissner's membrane towards the scala vestibuli after he had blocked the cochlear aqueduct in rabbits. Lindsay et al. (1952) successfully performed two such blockages in cats: after a time interval of four and eight months respectively, they found no histological changes in the inner ear. Uyama's finding may imply an excess of endolymphatic pressure as compared to the perilymphatic one, contrary to what is inferred by the results of Lindsay et al.

To our knowledge hitherto no one has measured the peri-, and endolymphatic pressures after blocking the cochlear aqueduct. We have performed this type of measurement: We observed time intervals of 1 hour and also of several weeks between blocking the aqueduct and the pressure measurement.

Materials and methods

First, proper localisation of the cochlear aqueduct is a necessity for a satisfactory blockage. Hereafter we had to drill a hole medio-caudal to the round window more or less perpendicular to the cochlear aqueduct. Blockage of this canal could then be achieved with dental cement.

Fig. 5–1 shows the path of the cochlear aqueduct made visible by the protruding ends of a therein inserted horse-hair. Approximately halfway the cochlear aqueduct the hole drilled in order to reach this canal is visible. Note the narrow margins of error one can allow oneself, when winding up at the right spot is to be assured. Quite some experience is required for drilling the hole at the right spot.

At some time after blockage the endolymphatic and perilymphatic pressures were measured as described in chapter III. Time intervals of either one hour or several weeks between obstructing the cochlear aqueduct and endolabyrinthine pressure measurement were observed. Whenever the elapsed time amounted to weeks, a sterile technique was employed during the first operation. Nevertheless, and in spite of additional prophylactic application of penicillin, inflammatory processes prohibited in several cases endolabyrinthine pressure measurements.

For comparison of the results, in several cases the pressures were measured on both sides, only one side having a blocked aqueduct.

Results

A survey of the results is presented in Table V–1. In each case the perilymphatic pressure at the side of the blocked cochlear aqueduct was inferior to the corresponding perilymphatic pressure at the non-obstructed side. Various influences could explain the spread in the perilymphatic pressure values after occlusion of the cochlear aqueduct. After depressurization of the perilymph via the three-way stopcock, in none of our cases a buildup to original values occurred if the cochlear aqueduct had been obstructed (see Table V–1). Whenever the cochlear aqueduct was intact a return to the original value occurred within two minutes.

Table V-1: Presentation of the values of the different parameters measured on the side where the cochlear aqueduct is blocked and where this aqueduct is patent.

	Identification number of the cat	5a	8a	9a	12a	4	9	11	14	16
	Time interval between obstruction of the cochlear aqueduct and pressure measurement (d=days and h=hours)	1h	1h	1h	1h	21d	21d	43d	58d	63d
↑ obstructed side	perilymph pressure (cm of water)	0.0	3.0	6.0	4.4 (0.0[1])	9.2 (1.2[1])	5.4	3.2	0.0	8.4 (3.2[1])
	difference between perilymphatic and endolymphatic pressure detectable[2]?	—	—	no	no	no	no	no	no	no
	breathing reflected?	—	—	—	no	no	—	no	no	no
	heartbeat reflected?	—	—	—	no	no	—	no	no	yes
↓	endolymphatic d.c. potential (mV)	—	—	78	96	84	78	90	72	96
↑ non-obstructed side	perilymph pressure (cm of water)	—	13	—	14.6	16.8 (16.8[1])	16.4 (16.4[1])	—	11.9	15.6 (15.6[1])
	ΔP in mm of water (perilymphatic pressure minus endolymphatic pressure)	—	—	—	0.0	−2.0	—	—	1.2	0.8
	breathing reflected?	—	—	—	yes	yes	—	—	yes	yes
	heartbeat reflected?	—	—	—	yes	no	—	—	yes	yes
↓	endolymphatic d.c. potential (mV)	—	—	—	96	84	90	—	72	96

[1]) Measurement two minutes after depressurization. (see text)

[2]) For measuring accuracy, see page 45.

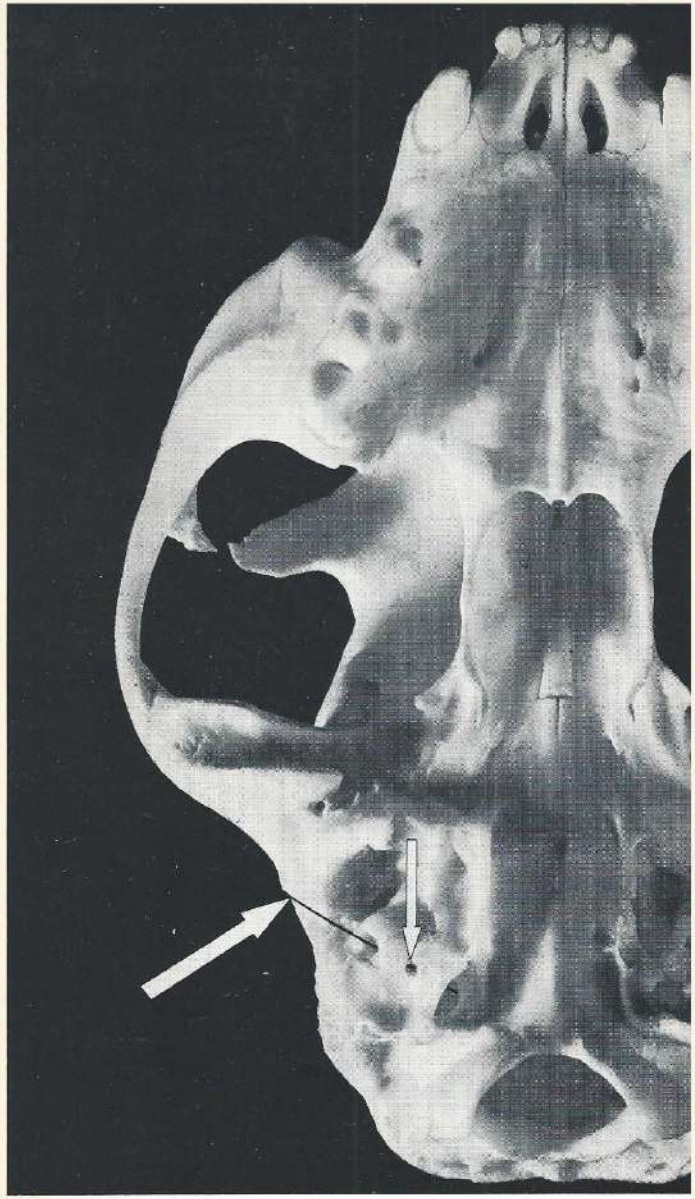

Fig. 5–1. The skull of a cat in which a hole (small arrow) was drilled perpendicularly to the cochlear aqueduct. A horse-hair (large arrow) was inserted for the purpose of checking.

Table V–1 indicates that we have found no detectable difference between endolymphatic and perilymphatic pressure. This finding was not as significant as in chapter III; the inexplicable fluctuations, being far more pronounced, caused a higher degree of inaccuracy.

The endolymphatic d.c. potentials appeared to be unaffected. Applying pressure to the abdomen, a procedure which normally would give a pressure increase of the perilymph and also of the endolymph, does no longer reveal itself after obstruction of the cochlear aqueduct; at the obstructed side pressure fluctuations reflecting breathing did not occur either, while fluctuations due to the heartbeat were seen in only one case.

No abnormal behaviour of the cats operated upon, which could point towards an abnormally functioning labyrinth, was observed.

CHAPTER VI

A. THE INFLUENCE OF DESTRUCTION OF THE ROUND WINDOW MEMBRANE ON THE ENDOLYMPHATIC PRESSURE

B. THE EXPANSION OF THE ENDOLYMPHATIC SPACE BY ARTIFICIAL MEANS UNTIL BREAKDOWN, PRESUMABLY OF REISSNER'S MEMBRANE

The fact that we found no difference between the endolymphatic and the perilymphatic pressure made us wonder whether a membrane, such as Reissner's, could uphold a pressure difference of any importance (some mm Hg as found by others (WEILLE et al. 1958, 1961 and MARTINEZ 1969)). After all, this thin two cell-layer membrane could prove too frail to maintain such a difference or to even stand it. The answer would be the more intriguing, since it could broaden our knowledge about Ménière's disease. Therefore we decided to pursue this matter further by opening the round window membrane, thus causing the perilymphatic pressure to become atmospheric. The following possibilities then present themselves:

1. The endolymphatic pressure will be partly affected or not at all.
2. The endolymphatic pressure will become atmospheric because of breakdown of the endolymphatic compartment.
3. The endolymphatic pressure will become atmospheric while the endolymphatic compartment remains intact.

In order to differentiate between these three possibilities, first the endolymphatic pressure had to be measured after partial removal of the secondary tympanic membrane. If the endolymphatic pressure became atmospheric we then would have to find out whether the endolymphatic compartment was still intact or not. We found the endolymphatic pressure to become atmospheric and the endolymphatic compartment to remain intact. This, however, did not give us an answer to the question as to how much pressure the endolymphatic compartment would take. All we had learned so far, was that presumably Reissner's membrane seems to stretch, thus enlarging the available space. Nonetheless we have been able to provide the answer to this last question by applying increasingly larger pressures to the endolymphatic compartment until rupture did occur.

HENRIKSSON et al. (1966) had previously carried out a study of this kind in the frog. He added a known amount of fluid to the endolymphatic system via a pipet in one of the semicircular canals and measured the resulting pressure via the same pipet. He determined the pressure as a

function of added volume and found the endolymphatic membranes to be elastic; at a pressure gradient of 5–8 cm of water or more frequently rupture occurred, mostly of the saccule. The endolymphatic membranes of the frog bear of course only a limited resemblance to those of the mammal in respect to both anatomy and histology.

Material and methods

In a cat the secondary tympanic membrane was partially removed, very carefully in order to avoid lesions in the immediate surroundings. Measurement of the endolymphatic pressure was performed in a manner analogous to the one described in chapter III. With the aid of a micromanipulator the tip of the microcannula (after the initial preparation of its distal end (see page 19)) was brought towards a point from which a perpendicular intersects with the basilar membrane at the side of the spiral ligament; this was now relatively simple, since the basilar membrane had become directly visible and more accessible (cf. page 20). In order to qualify, the measurement had again to fulfill the pertinent requirements outlined in chapter III.

On previous occasions we used the endolymphatic d.c. potential as an indicator of both the entrance of the microcannula tip into the endolymphatic space and of leakage. Also in these experiments the d.c. potential could at the same time indicate the intactness of the endolymphatic compartment.

Another indicator was utilized to the last mentioned purpose: Through the measuring microcannula tip trypan blue was brought into the endolymphatic space and the appearance of this colouring substance in the perilymphatic space was watched for through an operating microscope. Trypan blue has a molecular weight of 960.81 and supposedly will not penetrate the intact Reissner membrane, since the membrane is not permeable for thorium dioxyde (molecular weight 264.05 (Ilberg and Vosteen 1968)). Prior to the actual pressure measurement the microcannula, connective piece and measuring cannula were filled with blue coloured isotonic solution at the end distal to the isolating xylol (cf. Fig. 3–1). A pressure, slightly higher than the endolymphatic one, would cause the bluish solution to flow into the endolymphatic space. This pressure was applied via a fluid column in the calibration cannula by turning a three-way stopcock. Fig. 6–1 shows the experimental setup.

A sudden increase in the rate of flow served as a third indicator. We could determine the influx of fluid via the microcannula into the endolymphatic space by measuring the descent of the fluid level in the calibration cannula. With the exception of experiment number 20 (see Table VI–2) the ordinary calibration cannula was replaced by a polythene cannula of a smaller inner diameter, namely 1.5 mm, in order to ameliorate the measurement of the influx. In this chapter we will refer to this cannula as

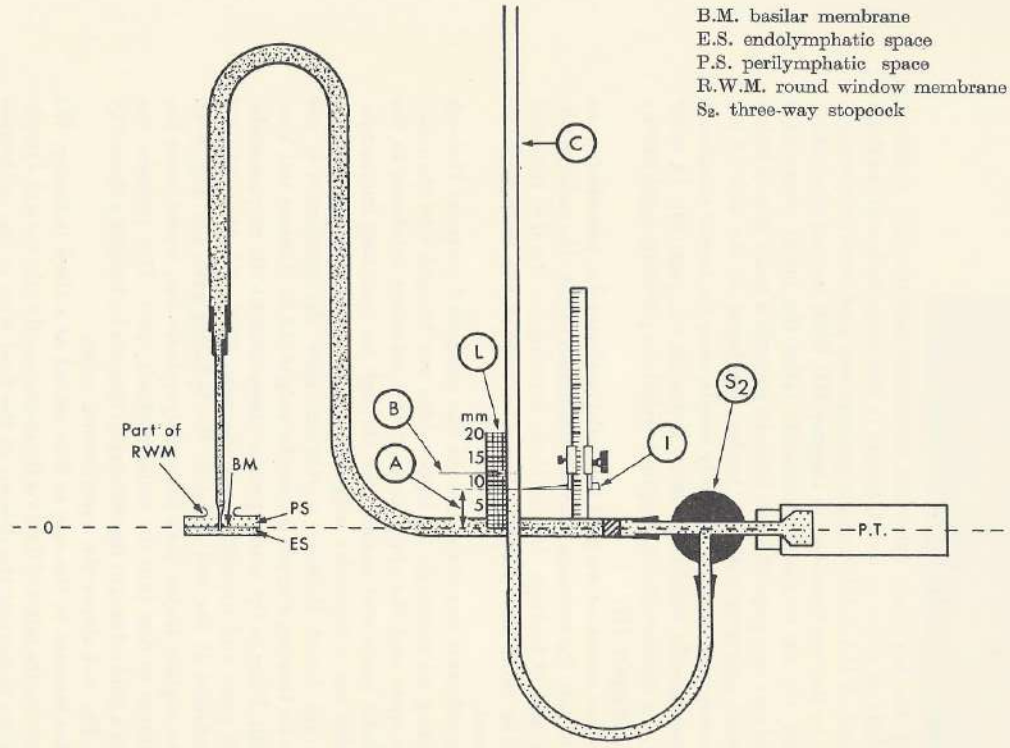

Fig. 6–1. Schematic drawing of the experimental setup to determine the maximum pressure until breakdown of the endolymphatic compartment, presumably because of rupture of Reissner's membrane. The pressure head, as a result of the water column A, causes influx of fluid into the endolymphatic compartment. The original position of the fluid in the pressure head cannula (C) is indicated by B (tiny horizontal arrow on linear graph paper (L) attached to C.) The level (indicator I) of the fluid above the zero line is maintained by raising the pressure head cannula with a micromanipulator (not shown). The influx can be calculated from the difference between B and I which is read from the linear graph paper or can be derived from the micromanipulator readings. The actual pressure head was measured with the pressure transducer (P.T.).

'pressure head cannula'. The applied overpressure was measured by the pressure transducer which was connected to the pressure head cannula and to the measuring cannula by means of the three-way stopcock. The height of the fluid column causing the pressure head could be increased by means of a second micromanipulator. Linear graph paper attached to the pressure head cannula made quantitative determination of the influx possible, as did the readings of the second micromanipulator. The height of the fluid column in the pressure head cannula over and above the measured endolymphatic pressure proved not to be a reliable indicator for measuring the over-pressure, because of relaxation phenomena, due to boundary layer tension. With the very small pressure heads under consideration in this chapter these phenomena could not be ignored, although for the calibration in previous chapters they were of no consequence. Unfortunately, our measurements were already completed when we discovered the discrepancy between the height of the fluid column and the pressure indicated via the transducer. Supplementary measurements in vitro have shown beyond doubt that the pressure transducer values were reliable. Fortunately, directly after each endolymphatic pressure measurement, we had turned the stopcock in order to connect the pressure transducer with the fluid column in the pressure head cannula bypassing the measuring cannula (for the endolymphatic pressure) in this procedure. Thereafter we altered the height of the fluid column – which had to provide a pressure head – in such a way that the pressure transducer showed exactly the same pressure value as previously obtained from the endolymph. The electromanometer was used at its highest sensitivity ranges (the range selector knob at position 10 or 20; see Fig. 3–4 and page 15). Not until after this action did the three-way stopcock take on its three-way connective function. We increased the pressure head stepwise in the course of each experiment. Each step lasted for some time during which the pressure head was maintained by keeping the fluid level more or less constant with the aid of the second micromanipulator, which had to be utilized because of fluid influx into the endolymphatic space.

We considered the question whether a small microcannula would require considerable time for the fluid to flow through it as a result of a large flow resistance. Our microcannula tips normally had outer diameters ranging from 30 to 130 microns. For the experiments described in this chapter we selected the larger cannulae to facilitate the fluid flow. They ranged from 110 to 130 microns (most of them were approximately 120 microns). A calculation after Poiseuille's law showed that the measured influx rates are not determined by the inner diameter of the microcannula tip. This can also be deduced from the sudden increase in flow rate when presumably Reissner's membrane ruptures. These rather large cannula tips need more precision in the localization of the right entrance spot of the basilar membrane. However, the partial removal of the round window membrane facilitates this task considerably.

As isotonic solution in the measuring cannula we employed either KCl (1.14%), NaCl (0.9%) or an endolymph-like substance consisting of:

NaCl 34 m Eq
KCl 114 m Eq
$NaHCO_3$ 32 m Eq
Dextran 1.1%
Aqua dest. ad 1000 ml.

Results

Not one in a series of 14 cases showed a difference between the pressures at either side of the basilar membrane; the perilymph and endolymph pressures were equal in all cases though not always precisely the same as the atmospheric pressure. This last finding is easily accounted for if one realizes the difficulties of placing the pressure transducer exactly on the right level; deviations amounted to some millimeters.

Endolymphatic pressure variations reflecting heartbeat (as observed on many previous occasions) or respiration while the round window membrane was intact, were completely absent in any test of this series in which the perilymphatic space was open.

No indication of a decrease in the endolymphatic d.c. potential as a result of the lowering of the perilymphatic pressure (and therefore also the endolymphatic one) to atmospheric level was found as is apparent from comparison of Table VI–1 with Table III–1.

Table VI-1. Values of the endolymphatic potentials as the endolabyrinthine pressure is made atmospheric by partial removal of the secondary tympanic membrane.

Experiment number	Endolymphatic potential in mV
4	87
6	90
7	96
8	87
9	102
11	102
13	87
14	96
15	90
16	90
17	87
18	90
19	102
20	108

After applying an over-pressure of 2 mm of water (or even less) with respect to the endolymphatic pressure, fluid enters the endolymphatic space as is concluded from column 4 of Table VI–2. This column also

shows that the endolymphatic compartment is obviously no longer intact after an influx of about 10 mm³. A small amount of fluid could theoretically have passed through the membrane. This may not be very consequential as the duration of the experiments seems to be of no influence on the quantity of influx till complete breakdown.

In this case the influx of approximately 10 mm³ of fluid would mean that the size of the endolymphatic space has increased by a factor of

Table VI–2. The total influx (influx) as a function of pressure and total time (time) of measurement.

Experiment number	Pressure head in mm of water (Pressure transducer values)	Time in minutes	Total influx in mm³	Isotonic fluid
13	5	10	8.5	NaCl 0.9
	12[2]	10.1[1] (+0.1)	10.2[3] (+1.7)	
14	2	2.5	1.7	NaCl 0.9
	6.8	5 (+2.5)	5.1 (+3.4)	
	11.4	7.5 (+2.5)	6.8 (+1.7)	
	16.4[2]	7.6[1] (+0.1)	8.5[3] (+1.7)	
15	2	2.5	2.5	NaCl 0.9
	5.4	4.83 (+2.33)	5 (+2.5)	
	11	7.58 (+2.75)	8.4 (+3.4)	
	16.4[3]	8.33[1] (+0.75)	10.1[3] (+1.7)	
16	0.5	10	0.9	NaCl 0.9
	3.5	19.75 (+9.75)	2.6 (+1.7)	
	8.5	30.25 (+10.5)	6.9 (+3.4)	
	12.5[2]	34.25[1] (+4)	9.4[3] (+3.4)	
18	5.2	3	—	KCl 1.14
	11.2	5.25 (+2.75)	—	
	15.2	21.75[1] (+16.5)	—	
	18.8[2]	immediately afterwards	10.3[3]	
19	0.8	9	1.7	KCl 1.14
	1.5	15.5 (+6.5)	4.3 (+2.6)	
	3.2	27 (+11.5)	6.9 (+2.6)	
	5.6[2]	31.5[1] (+4.5)	9.5[3] (+2.6)	
20	3	4.25	0.8	endolymph-
	6.25	7 (+2.75)	3.1 (+2.3)	like
	10.5[2]	19.75[1] (+12.75)	10.9[3] (+7.8)	

[1]) After this moment the influx rate suddenly became much higher due to breakdown, presumably of Reissner's membrane. This breakdown was confirmed shortly afterwards when we observed trypan blue invading the perilymph.
[2]) The upper limit which Reissner's membrane can stand.
[3]) Total influx up to the moment of sudden increase of influx rate.

about four compared to its original value (according to data given by MAGGIO (1966)).

Long before the actual rupture of the membrane the endocochlear d.c. potential has already reached a minimal value. As a matter of fact this potential decreases soon after influx has begun. It can be used to indicate that, at the start of the experiment, the membrane is intact. However, it cannot be used to determine the moment of rupture.

From Table VI–2 column 2, it can be seen that *the weakest wall of the endolymphatic space*, either Reissner's membrane or the wall of the saccule, *cannot stand a pressure of more than 2 cm of water*. In fact the pressure it can stand may be even less.

The duration of the experiments reported in Table VI–2 column 3 could perhaps have caused special changes in Reissner's membrane. Therefore, in two of our experiments we applied a pressure head of three cm of water to the endolymphatic space within a matter of minutes. Rupture was now observed after a time necessary for the passage of approximately 10 mm^3 through the microcannula tip.

Table VI–2 column 4 suggests that the influx rate is not directly proportional to the pressure increase. If one takes into account that bulging out of Reissner's membrane will probably be accompanied with an increase of the endolymphatic pressure, this would be understandable.

SUMMARY AND CONCLUSIONS

The histopathological picture in Ménière's disease has induced the idea of an endolymphatic hypertension; the histological picture of the normal labyrinth has likewise led to the hypothesis, held by several experts, of equal endolymphatic and perilymphatic pressures.

The outcome of extensive experimental work on the guinea pig by Weille et al. and by Martinez contradicted this last assumption entirely. These investigators found that in this animal the perilymphatic exceeds the endolymphatic pressure.

First of all it was our aim to resolve this controversy regarding the pressure gradient between perilymph and endolymph.

Like Weille et al. and Martinez we tried to measure in the guinea pig the endolymphatic and the perilymphatic pressure via the bony cochlear wall, employing a pressure transducer and an electromanometer.

We did not obtain reliable results in this manner and became convinced that the problems we encountered were insurmountable. We took a close look at some of these problems. Our own data and an analysis of the publications of Weille et al. and Martinez provided us with three causes which might, in retrospect, explain the erroneous finding of a higher perilymphatic pressure.

Ultimately, employing only one measuring system[1]), we succeeded in establishing the pressure gradient between the perilymph and endolymph in the cat and in one case in the guinea pig.

For this purpose the tip of a pyrex microcannula, which constitutes the most distal part of the measuring system, was allowed to pierce the round window membrane in order to measure perilymphatic pressure and, subsequently, to penetrate the basilar membrane at the side of the spiral ligament to measure endolymphatic pressure. Because this procedure occasions leakage of the endolabyrinthine fluids along the microcannula at the site of the secondary tympanic and the basilar membrane, posing a serious problem, a special technique had to be developed. Simultaneous measurement of the d.c. potential at the extreme end of the microcannula enabled us to establish the entrance of the microcannula tip into the endolymphatic space through the basilar membrane.

In both perilymph and endolymph similar pressure variations reflecting breathing were recorded. With our instrumentation it was possible in

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[1]) Determination of the pressure gradient between such fluids as perilymph and endolymph with two measuring systems creates great difficulty as detection of the minute pressure differences involved requires identical systems, which are hard to realise.

many cases to demonstrate superimposed heartbeat variations in these fluids as well. The pressure line was also subject to irregular pressure waves. At a later stage of our investigation corresponding pressure waves were also detected in the cerebrospinal fluid. The order of magnitude of the latter fluctuations was small, up to 3 mm of water, in an interval of variable duration, sometimes as short as 10 seconds. In 12 qualifying measurements out of some 80 cases, *the perilymphatic pressure* turned out to be exactly *equal* to *the endolymphatic pressure* within the limits of accuracy which are determined by our last mentioned irregular pressure waves. In these 12 measurements the absolute endolabyrinthine pressure was found to vary between 11.4 cm and 18.0 cm of water with an average value of 14.5 cm. The one successful sequential perilymphatic and endolymphatic pressure measurement in the guinea pig yielded also equal pressure values for these fluids.

Our way of measuring the endolabyrinthine pressure put forward a possibility to derive from micromanipulator readings, which distance the tip of the microcannula travelled from the moment of closing off the microcannula tip by the basilar membrane, until closing off occurred again, this time by Reissner's membrane. We used the phenomenon that the pressure fluctuations concordant with breathing and heartbeat disappear, when the basilar membrane and Reissner's membrane respectively close off the tip of the microcannula, recovering in between when these membranes have been perforated. It stands to reason that the point of perforation of the basilar membrane by the microcannula tip and the direction of the axis of this cannula determine the size of 'the distance'. The microcannula was directed nearly perpendicularly to the basilar membrane at the side of the spiral ligament. In this manner we measured the distance on 5 cats. Resulting values varied from 475 to 600 microns with an average value of 500 microns. Thus it has become possible to obtain an in vivo indication for the position of Reissner's membrane with respect to the basilar membrane. The d.c. potential is not suitable as a precise indicator of the encounter of the microcannula tip with the basilar and in particular Reissner's membrane; hence distance measurement cannot be reliably achieved using this parameter.

We also studied the relation between the pressures of the cerebrospinal fluid and endolabyrinthine fluids. In the 5 successful measurements the cerebrospinal fluid pressure appeared to be higher by an amount ranging from 0.2 to 1.7 cm of water with an average value of 0.8 cm. However, we cannot exclude the possibility of this being an artefact because of the usage of two measuring systems.

Application of an artificial overpressure of approximately 5 cm of water to the cerebrospinal fluid, resulted, after several minutes, in an equilibrium situation at which the cerebrospinal, the perilymphatic and the endolymphatic pressure had risen by the same amount. When this overpressure was not enforced any longer the pressure decrease was the same for all three fluids once equilibrium had been reached.

In the cerebrospinal fluid, pressure line fluctuations reflecting the respiration are clearly visible and far more pronounced than in the endolabyrinthine pressure line. Actually, these fluctuations in the endolabyrinthine pressure turned out to be an attenuated reflection of those in the cerebrospinal fluid: this we concluded from the observation that the endolabyrinthine pressure line looses the breathing fluctuations when we close off the cochlear aqueduct. The attenuation of the pressure fluctuations observed in the endolabyrinthine fluids is interpreted as a result of smoothing by the cochlear aqueduct with its small effective cross section; possible volume changes of the endolabyrinthine space due to a flexible round window membrane may contribute to this smoothing effect. Fluctuations due to heartbeat were also observed in the cerebrospinal fluid.

Application of abdominal pressure increases the cerebrospinal fluid pressure by a factor of up to two or three which is coupled to an equal rise in perilymphatic pressure. However, the perilymphatic pressure reaches its top slowly upon an abrupt rise in cerebrospinal fluid pressure; when abdominal pressure is stopped suddenly, the abrupt fall in cerebrospinal fluid pressure is accompanied by a slower but equal fall in perilymphatic pressure. This is equally applicable to perilymph and endolymph.

The small effective cross section of the cochlear aqueduct and possibly also changes in the volume of the endolabyrinthine space via the round window membrane may serve as a protective mechanism against consequences of sudden changes in the cerebrospinal fluid pressure.

With the purpose of blocking the cochlear aqueduct, in 9 cats we drilled a hole mediocaudal to the round window, this hole ending approximately half way the aqueduct. When the duct was reached it was obstructed with dental cement. The endolabyrinthine pressures were measured one hour after blocking in four of these cats and several weeks after blocking in the other five cats. We did not see any difference between these two groups. Normally, the endolabyrinthine pressure amounts to some 14 cm of water; after blocking the cochlear aqueduct this pressure was considerably lower, ranging from 0.0 cm to 9.2 cm of water with an average of 4.0 cm. In some cases we depressurized the perilymphatic compartment to atmospheric value. Ordinarily, when depressurization was discontinued the endolabyrinthine pressure recovered completely within minutes, but when we blocked the cochlear aqueduct, the endolabyrinthine pressure remained near atmospheric value. The fluctuations due to breathing were absent in all 'blocked' cases. Pressure fluctuations due to heartbeat seemed to occur in the endolabyrinthine fluid independent of the connection with the cerebrospinal fluid via the cochlear aqueduct. The vascularity of the endolabyrinthine space might explain this. The blocking operation had no effect on the endolymphatic potential. Also in these instances we did not detect any difference in pressure between the perilymph and endolymph.

We investigated whether the endolymphatic compartment remained intact when the round window membrane was partially removed and, if so, whether we could detect a pressure difference between the endolymph and the perilymph open to atmosphere. The endolymphatic compartment turned out to be intact, the pressure of the endolymph became atmospheric without showing pressure variations due to breathing or heartbeat; we found the endolymphatic potential to be unaffected.

Finally we determined in 7 cases after partial removal of the round window membrane, how much overpressure with respect to atmosphere could be applied to the intact endolymphatic compartment before rupture occurred. We increased the endolymphatic pressure in steps and applied each overpressure as long as inflow of fluid into the endolymphatic compartment was apparent and sometimes longer. The total duration of overpressure varied from 8 to 32 minutes with an average of 19. The overpressure before rupture of the endolymphatic compartment occurred was definitely lower than 2 cm of water. *Therefore, damage to the sensory cells due to pressure in case of an endolymphatic hypertension is very improbable provided the perilymphatic pressure is not affected, which is likely when the cochlear aqueduct is open.* The total amount of fluid which could enter the endolymphatic compartment before rupture was about 10 mm^3 implying a rather acute expansion of the endolymphatic compartment by a factor of four with respect to its original volume.

We may conclude that changes in the pressure of the cerebrospinal fluid are – with a time lag – reflected in the perilymph via the cochlear aqueduct and passed on to the endolymph, probably via Reissner's membrane. The hypothesis that the endolymphatic pressure is transmitted by the cerebrospinal fluid via the endolymphatic sac and duct appears to be refuted by our results.

Of course, whether our findings in the cat will be also applicable to man remains to be seen.

RESUME ET CONCLUSIONS

L'image histopathologique de la maladie de Ménière a donné naissance à l'idée d'hypertension endolymphatique, de même l'image histologique du labyrinthe normal suggère l'idée d'égalité de la pression de l'endolymphe et de la périlymphe. Il n'est donc pas étonnant que beaucoup d'oto-rhino-laryngologistes pensent que dans un labyrinthe normal la différence de pression entre la périlymphe et l'endolymphe est nulle.

Les résultats d'une recherche expérimentale approfondie aussi bien de Weille c.s. que de Martinez contredisent cette idée. Ils trouvèrent en effet que, chez les cobayes, la pression de la périlymphe était plus élevée que celle de l'endolymphe.

Notre premier objectif fût donc d'éclaircir cette controverse, concernant la différence de pression entre la périlymphe et l'endolymphe. Comme Weille c.s. et Martinez nous avons essayé de mesurer la pression, chez le cobaye, de la périlymphe et de l'endolymphe, à travers la cloison osseuse de la cochlée, à l'aide d'un „pressure transducer" et d'un électromanomètre. Les mesures de pression effectuées de cette façon ne nous ont pas donné de résultats dignes de foi, mais au contraire, nous ont convaincus que les problèmes soulevés par cette technique sont insurmontables. Nous avons ensuite étudié en détail un certain nombre de ces problèmes. Après avoir examiné les données de nos propres recherches, et analysé les publications de Weille c.s. et de Martinez, nous avons trouvé trois raisons pouvant peut-être expliquer pourquoi on a trouvé, à tort, que la pression de la périlymphe était plus élevée que celle de l'endolymphe.

Finalement, nous avons réussi à mesurer à l'aide d'un seul et même système[1]) la différence de pression entre l'endolymphe et la périlymphe, chez le chat et une fois, chez un cobaye. Pour mesurer la pression de la périlymphe, nous introduisons à travers la membrane de la fenêtre ronde, la pointe d'une microcanule en pyrex, qui est la partie la plus distale de notre système, puis nous l'introduisons à travers la membrane basilaire, du côté du ligament spiral pour mesurer la pression de l'endolymphe. L'infiltration des liquides labyrinthiques le long de la microcanule, à l'endroit où celle-ci traverse la membrane de la fenêtre ronde et la membrane basilaire, pose un grand problème, qui a été résolu par l'application d'une technique spéciale. En même temps, la mesure de la tension électrique, en courant continu, à la pointe de la microcanule, crée la possibilité de

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[1]) La détermination d'une différence de pression entre deux liquides comme la périlymphe et l'endolymphe, à l'aide de deux systèmes de mesure est extrèmement difficile, vu que la détermination d'une très petite différence de pression exige deux systèmes de mesure identiques, ce qui est pratiquement irréalisable.

déterminer le moment où cette pointe pénètre dans l'espace endolymphatique, à travers la membrane basilaire. Des fluctuations de pression dûes à la respiration, ont été enregistrées simultanément dans la périlymphe et dans l'endolymphe. De même notre matériel a permis d'enregistrer, dans ces liquides, des fluctuations de pression en concordance avec les battements du coeur.

La courbe d'enregistrement de la pression de la périlymphe et de l'endolymphe montre des ondes irrégulières, dont l'amplitude correspond à une pression de quelques mm d'eau (3 mm au maximum). A un stade plus avancé de nos recherches, nous trouvons des ondes de pression correspondantes dans le liquide cérébrospinal. La durée des ondes est variable, pas plus de 10 secondes parfois. Nous avons sélectionné selon des normes très sévères de mesures sûres, 12 expériences parmi les 80 effectuées. Il apparaît dans ces 12 cas, que *la pression de la périlymphe* est exactement *la même* que celle de *l'endolymphe*, abstraction faite des inexactitudes dûes aux ondes irrégulières ci-dessus nommées. Dans ces 12 mesures, la pression labyrinthique a varié de 11,4 cm à 18,0 cm d'eau avec une moyenne de 14,5 cm d'eau. Dans la seule mesure réussie sur le cobaye, la pression périlymphatique est aussi égale à la pression endolymphatique.

Cette façon de mesurer la pression labyrinthique a créé, en même temps, une possibilité de déterminer par la lecture d'un micromanipulateur, la distance parcourue par la pointe de la canule, du moment de fermeture de cette pointe, par la membrane basilaire, jusqu'au moment de sa fermeture par la membrane de Reissner. On a utilisé le fait que les fluctuations de pression en concordance avec la respiration et les battements du coeur, disparaissent quand la pointe de la microcanule est fermée par la membrane basilaire ou par la membrane de Reissner et reviennent au moment où ces membranes sont perforées. Naturellement, la distance mesurée dépend de l'endroit où la pointe de la microcanule perfore la membrane basilaire et de sa direction. La pointe de la microcanule est déplacée dans une direction à peu près perpendiculaire à la membrane basilaire, du côté du ligament spiral. On a mesuré de cette façon la „distance" sur 5 chats. Les valeurs trouvées varient de 475 microns à 600 microns avec une moyenne de 500 microns. On a donc créé de cette façon, la possibilité d'avoir, in vivo, des indications sur la position de la membrane de Reissner par rapport à la membrane basilaire. La tension électrique n'est pas un bon paramètre pour déterminer avec précision la rencontre de la pointe de la microcanule avec les membranes, surtout avec la membrane de Reissner. Pour cette raison, la détermination de la distance par les variations de tension électrique, n'est pas très sûre.

Nous avons aussi étudié la relation entre la pression du liquide cérébrospinal et celle des liquides labyrinthiques. Sur 5 cas étudiés, il apparaît que la pression du liquide cérébrospinal est plus haute de 0,2 à 1,7 cm d'eau que celle des liquides labyrinthiques, avec une moyenne de 0,8 cm. Cependant, il se peut que les différences de pression mesurées ne soient pas

l'enregistrement des phénomènes réels, car nous avons dû employer *deux* systèmes de mesure. Si, au moyen d'une colonne de liquide, on effectue sur le liquide cérébrospinal une surpression d'environ 5 cm d'eau, il en résulte, après quelques minutes, un état d'équilibre dans lequel la pression des liquides cérébrospinal, endolymphatique et périlymphatique est augmentée dans les mêmes proportions. Si on arrête cette surpression, les liquides, après avoir atteint leur équilibre, reprennent leur pression initiale. Les fluctuations de pression du liquide cérébrospinal en concordance avec la respiration sont bien visibles et beaucoup plus prononcées que dans les liquides du labyrinthe. En fait, les fluctuations de pression des liquides du labyrinthe sont une réflexion affaiblie de celles du liquide cérébrospinal. Nous sommes arrivés à cette conclusion: lorsque l'on ferme l'aqueduc cochléaire, la ligne d'enregistrement de la pression labyrinthique ne montre plus de fluctuations respiratoires. L'affaiblissement des fluctuations de pression du liquide cérébrospinal dans l'enregistrement de la pression des liquides labyrinthiques peut être expliqué par le très petit diamètre effectif de l'aqueduc cochléaire. Un changement possible de volume dans l'espace labyrinthique à cause de l'élasticité de la membrane de la fenêtre ronde, peut contribuer à cet affaiblissement. Des fluctuations dûes aux battements cardiaques ont été observées aussi dans le liquide cérébrospinal. En appuyant sur le ventre de l'animal, on peut faire monter la pression du liquide cérébrospinal du double ou du triple, ce qui entraîne une augmentation de même ordre de la pression du liquide labyrinthique. La pression de la périlymphe atteint lentement son maximum, tandis que celle du liquide cérébrospinal augmente brusquement. L'arrêt brusque de la pression sur le ventre est suivi d'un abaissement brusque de la pression du liquide cérébrospinal en même temps que d'un abaissement plus lent de la pression des liquides du labyrinthe et dans les mêmes proportions. Ceci est valable aussi bien pour l'endolymphe que pour la périlymphe. On peut considérer le très petit diamètre effectif de l'aqueduc cochléaire, et, peut-être aussi la possibilité de changement de volume de l'espace labyrinthique dû à l'élasticité de la membrane de la fenêtre ronde, comme une protection contre les variations soudaines de la pression du liquide cérébrospinal.

Nous avons percé, sur 9 chats, un trou du côté médio-caudal de la fenêtre ronde, pour boucher l'aqueduc cochléaire, ce trou étant situé à mi-chemin de l'aqueduc. L'aqueduc atteint, on le bouche avec du ciment dentaire. On a mesuré la pression des liquides labyrinthiques une heure après la fermeture, chez 4 chats, et plusieurs semaines plus tard, chez les 5 autres. On n'a trouvé aucune différence de mesure dans les deux groupes. La pression normale des liquides labyrinthiques est d'environ 14 cm d'eau. Après la fermeture de l'aqueduc, la pression est considérablement diminuée, variant de 0,0 à 9,2 cm d'eau, avec une moyenne de 4,0 cm. Dans quelques cas, nous avons amené à la pression atmosphérique l'espace périlymphatique. Normalement, dès que l'on cesse cette „dépres-

surisation", la pression labyrinthique reprend sa valeur initiale en quelques minutes, mais quand on ferme l'aqueduc cochléaire, la valeur de la pression labyrinthique oscille autour de celle de la pression atmosphérique. On n'a pas enregistré de fluctuations de la pression dûes à la respiration dans les cas „fermés". Les fluctuations de pression dûes aux battements cardiaques paraissent indépendantes d'une liaison avec le liquide cérébrospinal par l'aqueduc cochléaire. Ceci peut être expliqué par la vascularisation du labyrinthe. Dans les cas „fermés", nous n'avons remarqué aucune différence de pression entre périlymphe et endolymphe, pas plus qu'une influence sur la tension électrique endolymphatique.

Nous avons recherché si l'espace endolymphatique demeure intact après ablation partielle de la membrane de la fenêtre ronde, si oui, s'il existe une différence de pression entre l'endolymphe et la périlymphe ouverte à l'atmosphère. L'espace endolymphatique prend la valeur de la pression atmosphérique, sans montrer de fluctuations dûes à la respiration, ou aux battements cardiaques. La tension électrique de l'endolymphe reste inchangée.

Finalement, nous avons déterminé, dans 7 cas d'ablation partielle de la fenêtre ronde, de quelle valeur on peut augmenter la pression au dessus de celle de l'atmosphère, avant de provoquer une rupture de l'espace endolymphatique. Nous augmentons la pression par degrés, et, à chaque fois, nous attendons de ne plus voir de liquide pénétrer dans l'espace endolymphatique (nous attendons même parfois plus longtemps). La durée totale de l'augmentation de pression varie de 8 à 32 minutes avec une moyenne de 19 minutes. L'augmentation de pression nécessaire à la rupture est en tous cas inférieure à 2 cm d'eau. *C'est pourquoi il est improbable qu'une hypertension endolymphatique puisse endommager les cellules sensorielles, à condition que la pression périlymphatique reste normale, ce qui est vraisemblable quand l'aqueduc cochléaire est ouvert.* La quantité totale de liquide pouvant pénétrer dans l'espace endolymphatique avant la rupture est d'environ 10 mm^3 ce qui implique une augmentation plus ou moins soudaine d'environ 4 fois son volume initial.

Les changements de pression dans le liquide cérébrospinal sont transmis à la périlymphe par l'aqueduc cochléaire et vraisemblablement retransmis à l'endolymphe par la membrane de Reissner. Nos résultats de mesure rejettent l'hypothèse d'une transmission de la pression du liquide cérébrospinal à l'endolymphe, par le sac et le conduit endolymphatique.

Bien sûr, on peut se demander si ces découvertes chez le chat sont applicables à l'homme.

SAMENVATTING EN CONCLUSIES

Evenals het histopathologische beeld bij de ziekte van Ménière heeft geleid tot het begrip „endolymfatische hypertensie", zo suggereert het histologische beeld van het normale labyrint een gelijke druk van perilymfe en endolymfe. Het is daarom niet verwonderlijk dat vele otologen de mening zijn toegedaan, dat in het normale labyrint de drukgradiënt tussen perilymfe en endolymfe nul is.

De resultaten van een uitgebreid experimenteel onderzoek van zowel Weille c.s. als van Martinez zijn hier echter mee in tegenspraak. Zij vonden dat bij de cavia de druk van de perilymfe hoger was dan die van de endolymfe.

In eerste instantie was het onze opzet deze controverse betreffende de drukgradiënt tussen perilymfe en endolymfe weg te nemen. Evenals bovengenoemde onderzoekers hebben wij getracht bij de cavia de druk van perilymfe en endolymfe te meten via de benige cochleawand met behulp van een „pressure transducer" en een electromanometer. Drukmetingen op een dergelijke wijze uitgevoerd, hebben ons geen betrouwbare resultaten verschaft, maar ons de overtuiging gegeven dat de problemen die zich hierbij voordoen, onoverkomelijk zijn. Een aantal van deze problemen hebben wij nader onder de loep genomen. Gegevens van eigen onderzoek en een analyse van de publicaties van Weille c.s. en Martinez hebben ons drie oorzaken verschaft, die wellicht verklaren, waardoor de perilymfe druk – achteraf gezien verkeerdelijk – hoger werd bevonden dan de endolymfe druk.

Uiteindelijk zijn wij erin geslaagd de drukgradiënt tussen perilymfe en endolymfe bij de kat – en in één geval ook bij de cavia – te meten met behulp van slechts één meetsysteem[1]). Hiertoe doorboorden wij met de punt van een pyrex microcanule, die het meest distale deel van het meetsysteem vormde, de ronde venster membraan voor meting van de perilymfe druk en vervolgens de basilaire membraan aan de kant van het ligamentum spirale voor meting van de endolymfe druk. Hierbij vormde lekkage van labyrintvloeistof langs de microcanule ter plaatse van de ronde venster membraan en de basilaire membraan een groot probleem, dat werd opgelost door toepassing van een speciale techniek.

Het tegelijkertijd meten van de gelijkspanning aan de punt van de microcanule maakte het mogelijk vast te stellen wanneer deze punt de

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[1]) Bepaling van een drukverschil tussen vloeistoffen als perilymfe en endolymfe met twee meetsystemen is uiterst moeilijk, aangezien het vaststellen van een zeer kleine drukgradiënt, meetsystemen vereist die identiek zijn, hetgeen ternauwernood te verwezenlijken is.

endolymfe ruimte via de basilaire membraan binnendrong. Drukschommelingen, de ademhaling weerspiegelend, werden gelijkelijk in perilymfe en endolymfe geregistreerd. Onze instrumentatie maakte het veelal mogelijk tevens drukfluctuaties corresponderend met de hartslag in deze vloeistoffen vast te stellen. De registratielijn van de druk van perilymfe en endolymfe vertoonde ook onregelmatige golven waarvan de amplitude overeenkwam met een druk van enige (tot een maximum van 3) millimeters water. Corresponderende drukgolven deden zich, zoals later bleek, ook in de liquor cerebrospinalis voor. De duur per golf was variabel, soms niet langer dan 10 seconden. Van een 80-tal experimenten voldeden 12 aan de strenge eisen door ons gesteld voor een betrouwbare meting. In deze 12 gevallen bleek *de perilymfatische druk* precies *gelijk* te zijn aan *de endolymfatische*, afgezien van de onnauwkeurigheid veroorzaakt door laatstgenoemde onregelmatige drukgolven. In deze 12 metingen varieerde de labyrintdruk van 11,4 tot 18,0 cm water, met een gemiddelde waarde van 14,5 cm. Ook in de enige bij de cavia geslaagde meting, bleek de perilymfatische druk gelijk te zijn aan de endolymfatische.

De wijze waarop wij metingen van de labyrintdruk hebben verricht, schonk ons tevens een mogelijkheid om uit aflezingen van een micromanipulator te bepalen, welke afstand de punt van de microcanule aflegde vanaf het moment van afsluiting van deze punt door de basilaire membraan totdat wederom afsluiting plaats vond, ditmaal door de membraan van Reissner. Er werd gebruik gemaakt van het feit dat de drukfluctuaties corresponderend met de ademhaling en hartslag verdwijnen wanneer respectievelijk de basilaire membraan en de membraan van Reissner de punt van de microcanule afsluiten, en terugkeren wanneer deze membranen eenmaal geperforeerd zijn. Uiteraard bepalen de plaats van de perforatie van de basilaire membraan door de punt van de microcanule en de richting van de canule-as de grootte van „de afstand". De punt van de microcanule werd verplaatst in een richting bijkans loodrecht op de membrana basilaris, aan de kant van het ligamentum spirale. Een dergelijke afstand werd bij 5 katten gemeten. De gevonden waarden varieerden van 475 tot 600 micron, met een gemiddelde van 500 micron. Hiermee is dus een mogelijkheid geschapen om in vivo een aanwijzing te verkrijgen voor de stand van de membraan van Reissner ten opzichte van de basilaire membraan. De gelijkspanning is geen goede parameter om de ontmoeting van de punt van de microcanule met de membranen precies aan te geven, speciaal indien deze de membraan van Reissner betreft. Bepaling van „de afstand" is daardoor niet erg betrouwbaar wanneer we op de gelijkspanning afgaan.

We hebben ook het verband tussen de drukken van de cerebrospinale vloeistof en de labyrintvloeistoffen bestudeerd. De druk in de cerebrospinale vloeistof bleek, zoals gemeten werd in 5 gevallen, 0,2 tot 1,7 cm water hoger te zijn dan die in de vloeistoffen van het labyrint, met een gemiddelde waarde van 0,8 cm. De mogelijkheid dat deze drukverschillen op een

artefact berusten, kan echter niet worden uitgesloten, doordat wij 2 meetsystemen hebben gebruikt. Wanneer we door middel van een vloeistofkolom een overdruk van ongeveer 5 cm water uitoefenden op de liquor cerebrospinalis, resulteerde dit na enige minuten in een evenwichtstoestand, waarbij de liquor, de perilymfe en endolymfe drukken in dezelfde mate gestegen waren. Wanneer de overdruk op de cerebrospinale vloeistof werd opgeheven, bleek de daling van de druk in deze en in de labyrintvloeistoffen, na het bereiken van een evenwicht, even groot te zijn. In de liquor cerebrospinalis waren de fluctuaties in de druk corresponderend met de ademhaling zeer duidelijk zichtbaar en veel sterker geprononceerd dan in de labyrintvloeistoffen. In feite bleken de drukfluctuaties van de labyrintvloeistoffen een verzwakte weergave te zijn van die in de liquor. Tot deze conclusie zijn wij gekomen op grond van het feit dat, indien we de aquaeductus cochlearis hadden afgesloten, de registratielijn van de labyrintdruk geen ademhalingsfluctuaties meer vertoonde. Verzwakking van drukfluctuaties in de liquor cerebrospinalis, zoals die werden waargenomen in de labyrintvloeistoffen, kan worden uitgelegd als te zijn veroorzaakt door de uiterst kleine effectieve doorsnee van de aquaeductus cochlearis; een mogelijke volumeverandering van de labyrintruimte door meegeven van de membraan van het ronde venster zou eventueel kunnen bijdragen tot deze verzwakking. Ook in de liquor cerebrospinalis werden drukfluctuaties corresponderend met de hartslag geregistreerd. Door druk op de buik uit te oefenen konden wij de druk van de liquor met een factor 2 tot 3 doen stijgen, hetgeen resulteerde in een even grote drukstijging van de perilymfe. De perilymfe druk bereikte zijn maximum langzaam, terwijl de drukstijging van de liquor cerebrospinalis abrupt geschiedde; een plotseling opheffen van de druk op de buik had een snelle daling van de druk in de liquor tot gevolg, terwijl de perilymfe druk trager maar in gelijke mate daalde. Dit bleek niet alleen voor de perilymfe druk te gelden, maar ook voor de endolymfe. De uiterst kleine effectieve doorsnee van de aquaeductus cochlearis en wellicht ook de mogelijkheid tot volumeverandering van het labyrint door meegeven van de ronde venster membraan kunnen gezien worden als een beschermingsmechanisme tegen de gevolgen van plotselinge veranderingen in de druk van de liquor cerebrospinalis.

Bij 9 katten hebben we, met de bedoeling afsluiting van de aquaeductus cochlearis tot stand te brengen, een gat geboord medio-caudaal van het ronde venster, zó dat we ongeveer halverwege deze ductus uitkwamen. Vervolgens werd de aquaeductus cochlearis met tandartsencement afgesloten. De druk van de labyrintvloeistoffen werd bij 4 katten 1 uur na afsluiting gemeten en bij de overige 5 een aantal weken later. Wat betreft de meetresultaten vonden we geen verschil tussen deze 2 groepen. Normaliter ligt de labyrintdruk om en nabij 14 cm water. Na afsluiting van de aquaeductus cochlearis was deze drukwaarde aanzienlijk lager, namelijk varierend van 0,0 cm tot 9,2 cm water, met een gemiddelde van 4,0 cm. In een aantal gevallen hebben we de druk in de perilymfe ruimte atmos-

ferisch gemaakt. Indien blootstelling van de perilymfatische ruimte aan de atmosfeer werd beëindigd, herstelde de labyrintdruk zich gewoonlijk binnen enige minuten geheel, maar wanneer de aquaeductus cochlearis was afgesloten, bleef de labyrintdruk nabij atmosferische waarde. Schommelingen in de druk de ademhaling weerspiegelend, ontbraken in alle „afgesloten" gevallen. Hartslagfluctuaties in de druklijn leken onafhankelijk van een verbinding met de liquor cerebrospinalis via de aquaeductus cochlearis op te kunnen treden in de labyrintvloeistoffen. De vascularisatie van het labyrint zou dat kunnen verklaren. We hebben geen drukverschil tussen perilymfe en endolymfe kunnen aantonen in geval van een afgesloten aquaeductus cochlearis, noch een beinvloeding van de endolymfatische gelijkspanning.

Wij gingen ook na of de endolymfatische ruimte intact bleef, wanneer we de ronde venster membraan gedeeltelijk verwijderden en of wij, indien dit het geval was, een drukverschil konden aantonen tussen de endolymfe en de aan atmosfeer blootgestelde perilymfe. De endolymfatische ruimte bleek intact te zijn gebleven terwijl de endolymfe druk eveneens atmosferisch was geworden zonder drukfluctuaties corresponderend met de ademhaling of de hartslag te vertonen. De endolymfatische gelijkspanning daarentegen bleek onbeinvloed.

Tenslotte hebben we in 7 gevallen na gedeeltelijke verwijdering van de membraan van het ronde venster bepaald hoeveel overdruk ten opzichte van de atmosfeer op de intact gebleven endolymfatische ruimte kon worden gezet, alvorens deze barstte. Een dergelijke overdruk werd stapsgewijs bereikt, waarbij iedere drukverhoging werd volgehouden zolang er vloeistof in de endolymfatische ruimte leek binnen te stromen en soms langer. De totale tijd gedurende welke overdruk werd gegeven, varieerde van 8 tot 32 minuten met een gemiddelde van 19 minuten. De overdruk, die uitgeoefend kon worden op de endolymfatische ruimte voordat deze barstte, was beslist lager dan 2 cm water. *Daarom is beschadiging van het zintuigepitheel tengevolge van druk in geval van een endolymfatische hypertensie erg onwaarschijnlijk, vooropgesteld dat de perilymfatische druk onberoerd is gebleven, hetgeen bij een open aquaeductus cochlearis waarschijnlijk is.* De totale hoeveelheid vloeistof die de endolymfatische ruimte kon binnenstromen alvorens deze barstte, bedroeg ongeveer 10 mm^3; dit zou een betrekkelijk plotselinge vergroting met een factor 4 van de endolymfatische ruimte betekenen.

Wij zijn tot de conclusie gekomen dat veranderingen in de druk van de cerebrospinale vloeistof worden overgebracht naar de perilymfe via de aquaeductus cochlearis en worden doorgegeven aan de endolymfe, waarschijnlijk via de membraan van Reissner. De hypothese dat de druk van de cerebrospinale vloeistof aan de endolymfe wordt doorgegeven via de saccus en ductus endolymphaticus lijkt door onze meetresultaten weerlegd te zijn.

In hoeverre onze bevindingen bij de kat gelden voor de mens, blijft een open vraag.

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STELLINGEN

I.

Indien een operatief of tandheelkundig ingrijpen bij een patiënt met een hartgebrek prophylactisch parenterale toediening van een antibioticum vereist, dient deze toediening niet langer dan één uur voor de ingreep aan te vangen.

II.

Bij kinderartsen zou een grotere bereidheid moeten bestaan zieke vroeggeborenen vanuit de periferie naar een goed geoutilleerde afdeling voor pasgeborenen over te plaatsen.

III.

De kennis betreffende de onderlinge beïnvloeding van gelijktijdig toegediende geneesmiddelen is nog volkomen ontoereikend.

IV.

Geen kind behoort tot welke vorm van B.L.O. dan ook te worden toegelaten zonder dat onderzoek van zijn gezichtsvermogen en gehoor heeft plaats gevonden.

V.

De overheid dient die maatregelen te treffen, die noodzakelijk zijn om een verantwoord standpunt te kunnen innemen in haar wettelijk beleid ten opzichte van de zg. „soft drugs" hasjiesj en marihuana, om zodoende de bestrijding van „hard drugs" effectiever ter hand te kunnen nemen.

VI.

Men zou een beengeleidingscurve, die gemeten is zonder voldoende maskering, dienen te noteren met een teken waaraan de betrekking op het linker, respectievelijk rechter oor ontbreekt.

VII.

Om van een juiste dosering van fenytoinum aan epilepsie patiënten verzekerd te zijn is bepaling van de serumspiegel van dit medicament een conditio sine qua non.

VIII.

Het D.N.A., hetgeen Bell in het cytoplasma van spierweefsel van kippenembryo's meende waar te nemen (Nature 224/10, 326–328, 1969) is een artefact teweeggebracht door de wijze van prepareren. De theorie dat dit D.N.A. informatie overdraagt van kern naar cytoplasma, is daarom niet langer houdbaar.

IX.

Bij het routine keel-, neus- en oorheelkundig onderzoek is de enig psychologisch verantwoorde volgorde: oor, neus, keel.

X.

De conclusie, afgeleid uit de gegevens van Anon (Nature 226/5, 869–870, 1970), dat de baardgroei zou toenemen bij spoedig te verwachten sexuele activiteit na een abstinentieperiode, is onvoldoende gefundeerd.

XI.

In het kader van het aanschouwelijk onderwijs dient aan zulk een phenomeen als de neiging tot oprispen na het drinken van koude koolzuurhoudende dranken beslist aandacht gegeven te worden ter demonstratie van de temperatuurafhankelijkheid van de evenredigheidsconstante in de wet van Henry.