BIOPHYSICS OF THE SENSE OF SMELL

M. STUIVER

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STELLINGEN

1

Door de variaties in de hoeveelheid zonnestraling op het aardoppervlak te correleren met de door middel van de O^{18}/O^{16} verhouding bepaalde temperatuurvariaties in diepzee-afzettingen, komt Emiliani tot een grafiek waarin de temperatuurvariaties voor de laatste 300.000 jaar als functie van de tijd gegeven worden. Tegen de wijze waarop dit verband gelegd wordt zijn ernstige bezwaren aan te voeren.

Emiliani, C.; J. of Geology **63** (1955) 538.

2

α lijnen van natuurlijke radioactieve bronnen, gemaakt volgens de klassieke verzamelmethode van O. Hahn, vertonen een grotere asymmetrie dan verwacht werd. Deze asymmetrie kan op eenvoudige wijze verklaard worden.

3

Tegen de wijze waarop Peierls een verband tracht te vinden tussen het kollectieve model van de atoomkern en de beschrijving van kernkrachten als meer-deeltjes probleem, zijn bezwaren aan te voeren.

Peierls, R.E. en Yoccoz, J.; Proc.phys.Soc. **70** (1957) 381.
Peierls, R.E.; Proc. of the Rehovoth conference blz. 395.

4

De verklaring van het reukmechanisme, zoals deze door Moncrief wordt gegeven, berust op een onjuiste interpretatie van het resonantie begrip in de organische chemie.

Moncrief, R.W.; The chemical senses (1951) blz. 395.

5

Een herhaling van de door Beck en Miles uitgevoerde experimenten is noodzakelijk om te onderzoeken of de door hen opgestelde reuktheorie inderdaad in bepaalde gevallen geldig is.

Miles, R.W. en Beck, L.H.; Science **106** (1947) 512.

6

Tegen de wijze waarop Versteeg tot de conclusie komt dat de adaptatie een perifeer verschijnsel is, zijn bezwaren aan te voeren.

Versteeg, N.; dissertatie 1956.

7

De conclusie welke Le Magnen trekt uit de door Woodrow en Karpman gevonden relatie tussen de sterkte van de adapterende prikkel en de tijd, nodig voor het verdwijnen van de reuksensatie, is onjuist.

Le Magnen, J.; J. Physiologie **45** (1953) 285.

8

De experimenten van Moncrief, wederzijdse adaptatie betreffende, verliezen veel aan waarde doordat enkele essentiele grootheden niet bepaald zijn.

Moncrief, R.W.; J. of Physiology **133** (1956) 301.

9

Het verdient aanbeveling om in het leerprogramma van de middelbare scholen een aantal natuurkunde proeven op te nemen welke gedemonstreerd *moeten* worden.

10

Het verdient aanbeveling om naast de jacht- en visacte een acte voor het rapen van kievietseieren (z.g. „raapacte") in te voeren.

RIJKSUNIVERSITEIT TE GRONINGEN

BIOPHYSICS OF THE SENSE OF SMELL

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKSUNIVERSITEIT TE GRONINGEN OP GEZAG VAN DE RECTOR MAGNIFICUS DR. F. H. L. VAN OS, HOOGLERAAR IN DE FACULTEIT DER WIS- EN NATUURKUNDE. TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE TE VERDEDIGEN OP DINSDAG 29 APRIL 1958 DES NAMIDDAGS OM 3 UUR

DOOR

MINZE STUIVER

GEBOREN TE VLAGTWEDDE

UITGEVERIJ EXCELSIOR - ORANJEPLEIN 96 - 'S-GRAVENHAGE

Promotor: Prof. Dr. Hl. de Vries

Aan mijn Ouders
Aan Anneke

CONTENTS

INTRODUCTION

Among the biological systems the sense organs are very attractive from a physical point of view because of their similarity to instruments. In connection with the investigations concerning the physical aspects of the sense organs, it seemed important to investigate the sense of smell and taste next to vision, hearing and other labyrinth organs. As for taste stimulus control seems to be difficult, the sense of smell was apparently the most suitable of the chemical senses. The investigations of the physical, as well as the physiological and biochemical aspects of the sense organ have been much more extensive for the sense of sight and hearing than for the sense of smell. This may be partly caused by lack of interest, but undoubtedly also on account of experimental difficulties.

One of the most serious difficulties for the investigation of the sense of smell is that only a small number of observations can be made during a certain period of time because otherwise adaptation will occur. As the experiments take a long time, fluctuations in the sensitivity of the subject are likely. So the experimental results always scatter and a large number of experiments is necessary to obtain exact results.

Several criticisms are applicable to the equipments used for smell investigations. Our first aim was the development of an apparatus with an adequate stimulus control. A description of the equipment is given in Chapter I.

With the apparatus constructed, it is possible to study the absolute sensitivity of the sense organ and the influence of statistical fluctuations in the number of molecules striking the olfactory epithelium upon the frequency of smelling curve. Analogous investigations in vision by various investigators resulted in the determination of the number of quanta to which one rod responds and the number of rods which have to act together simultaneously for a sensation to be perceived.

In order to calculate the absolute sensitivity of the sense of smell it is necessary to determine the conditions in which the number of molecules necessary to evoke a smell sensation is as small as possible. For that purpose the influence of the stimulus

duration and the rate of flow upon the threshold has been determined. (Chapter II and III.) The ratios of the experimental threshold concentrations for different rates of flow are compared to the ratios deduced from a theory which takes into account diffusion and absorption phenomena in the nose.

From the influence of the statistical fluctuations in the number of molecules striking the olfactory epithelium upon the frequency of smelling curve it is possible to draw some conclusions about the receptor mechanism. (Chapter III).

Like other sensory systems the olfactory sense displays the phenomenon of adaptation. The influence of stimulus intensity and adaptation time upon the threshold will be discussed in Chapter IV. The peripheral receptor mechanism appears to be only to a small extent responsible for the adaptation process.

A problem still unsolved is the relation between threshold and chemical structure. The influence of steroisomerism upon the threshold and the variations of the threshold for a series of ortho-, meta- and para-compounds will be discussed in chapter V.

Chapter I

DESCRIPTION OF THE APPARATUS

1.1. *Techniques in olfactometry*

A critical review of the techniques in olfactometry has been published by Wenzel (1). We shall discuss here the techniques which are related to our apparatus or which have been used to study the same phenomena as discussed in this thesis.

The apparatus of most frequent use for the determination of smell thresholds is the olfactometer, an instrument designed by the Dutch physiologist Zwaardemaker. It consists of a glass tube, open at both ends over which another tube is loosely slipped. The inner surface of the outer tube has been impregnated with an odorous material. When a small part of the outer tube is covered by the inner tube, a large number of odorous molecules can escape into the glass tube and into the nose of the subject when sniffing. For a standardized olfactometer the olfactie is the olfactory unit. This unit is defined as the normally perceptible minimum odour and stated in terms of exposure of the odorous substance in cm.

This technique is subject to several criticisms. Adhesion to the inner tube is important. The temperature of the odorous material is uncontrolled and the possibility exists that the subject influences the threshold by the volume of his sniff. Zwaardemaker designed another type to meet the latter difficulty. A small pump was attached to the instrument, forcing air at a constant rate through the apparatus.

The olfactometer is based on the assumption that the number of molecules released by the odorous substance, is proportional to the area exposed. As Woodrow and Karpman (2) pointed out already, this assumption is only true if the air does not become saturated. If saturated, enlarging of the area of the odorous substance will have no influence upon the number of molecules delivered to the subject.

Notwithstanding these objections, the olfactometer, compared with the other techniques, is fairly reliable. This is evident

from some results obtained by Komuro with adaptation measurements (3). With the aid of the olfactometer he measured the sensitivity to terpinol after previous adaptation. The adaptation occurred in a box with a capacity of 400 liters. Certain quantities of odorous material had been evaporated in this box previously. After complete adaptation in this box the raised threshold of the subject, expressed in olfacties, proved to be proportional to the weight of the evaporated odorous substance. As for complete adaptation the raising of the threshold is nearly proportional to the adapting intensity (see 4.5), we may conclude that the number of molecules given off by the odorous substance is about proportional to the area exposed. Of course it is possible that for other substances the area exposed is not proportional to the number of molecules delivered to the subject. Also there remains the difficulty that it is impossible to express the threshold in molecules per cc.

There still exists a group of investigators using a method which is certainly not commendable. This method consists of smelling at flasks containing at the bottom different amounts of odorpus substance dissolved in a liquid. The threshold for smelling is then expressed in the concentration of the odorous substance in the liquid. However, the partial vapour pressure of the odorous substance is not always proportional to the amount of substance dissolved. Some tables in Landolt-Bornstein (4) give data of the partial vapour-pressure and the amount of substance dissolved in some specific liquids. The determination of the partial vapour pressures is required when this method is used. This has never been done in smell experiments. Adhesion of odorous molecules to the walls of the flasks is also an important factor. The method is only useful for very rough experiments, therefore, which are yet successful in a field as unexplored as the sense of smell.

The technique of Elsberg and Levy (5) is very important. With this method an odorous liquid is poured into a 500 cc bottle. An outlet tube leads from the bottle to the nostrils; an inlettube is attached to a hypodermic syringe. A pinchcock on the outlet tube is kept closed while a few cubic centimeters of air is forced into the bottle. When the pinchcock is suddenly removed, a blast of odorous vapour comes into the nostrils.

However, the technique has several imperfections. During injection the rate of flow of the injected air is not constant, but changes from a maximum value to zero; this time course of change

is different for each stimulus, because it depends on the speed with which the pinchcock used is removed. Another difficulty is that the number of odorous molecules blast into the nostrils, can only be changed by varying the rate of flow. Jerome (6) has studied the influence of variation of volume and rate of flow upon the olfactory thresholds for the method mentioned above.

The determination of olfactory thresholds with the Elsberg method seems to be so inaccurate that Jones (7) was not able to demonstrate a change in threshold when he used the same "rate of flow" and volume but different odour concentrations.

Wenzel improved upon the original technique of Elsberg by arranging for uniform pressure to be exerted throughout the duration of a given exposure. In her review she concludes that the most significant characteristics are the control of the rate of flow, duration of the stimulus and the number of molecules of the odorous substance in each stimulus. These requirements were only met by the technique developed by Wenzel. It appeared desirable, however, to modify these requirements into control of the rate of flow, duration of stimulus and number of odorous molecules so that each one can be varied independently of the other. This modified requirement is not met by Wenzel. For a constant duration of the stimulus she could only change the number of injected odorous molecules by changing the rate of flow. Variations in the rate of flow, however, make a differential appeal to the cutaneous receptors of the nostrils and introduce a second variable.

Summarizing we can say that only a technique with an independent control of duration of the stimulus, the rate of flow and the number of odorous molecules satisfies the requirements necessary for an adequate determination of olfactory thresholds.

1.2. *The apparatus*

An important problem is the removal of impurities in the air used for the determination of thresholds. As was pointed out already by Foster a.o. (8), it is impossible to use charcoal to remove impurities because the carbon itself is not odorless. We had the same experience when using silicagel. Heating the silicagel up to 100°C and pumping off the vapours during several hours gives some slight, but not sufficient, improvement.

It is also possible to purify the air by leading it through a Cottrel apparatus. However, a large amount of odorous ozone is produced in this equipment. It is necessary to use chemical sub-

stances for removing the ozone. We experienced that chemical substances always introduce more or less new contaminations. Further the used rates of flow (upto 300 cc per second) also require a large equipment. For very small rates of flow (e.g. 10 cc per second) the use of liquid air for the condensation of impurities is recommended. No new contaminations are introduced and the impurities are removed to a large extent. However, this method is too expensive for larger rates of flow because large quantities of liquid air are required.

The flow of air is supplied by a rotating compressor, which has been cleaned carefully and which is lubricated with paraffin oil. When the compressor had been delivered, the air supplied was strongly contaminated with oily and other smells. For cleaning, the whole compressor was disassembled. The used lubricant was dissolved by placing all parts of the compressor twice or more often in pure ether. We also used alcohol for this purpose. Some of the inner metal parts of the compressor had been covered by the manufacturer with a kind of aluminium paint. This paint was removed with a concentrated sodium hydroxyde solution. After this, all parts of the compressor were heated to about 100°C and placed in a vacuumchamber. The vapours given off from the accessories of the compressor were pumped away. The entire procedure was repeated several times.

In our case it proved to be sufficient to use only a device for washing the air. The air coming through the apparatus is only then pure if the air outside is not contaminated. During the experiments a well-trained subject can immediately notice contaminations coming from outside, the experiments are then stopped for a few minutes. The air outside the laboratory is quite pure due to the position of the laboratory. Only a few times a week the air appeared to be slightly contaminated.

The lubrication of the compressor with paraffin oil proved to be successful. However, if the compressor has been used for about a hundred hours, some oily contamination will be originated. This contamination is caused by an oxydation product of the paraffin oil (working temperature of the compressor is about 80°C). It is necessary to clean the entire compressor then.

We shall now discuss the apparatus, which fulfills the requirements mentioned in 1.1. A diagram of the main features is shown in fig. 1*a*.

Essentially, the apparatus has three sections. The upper line in figure 1*a* carries the main stream of air which is supplied to

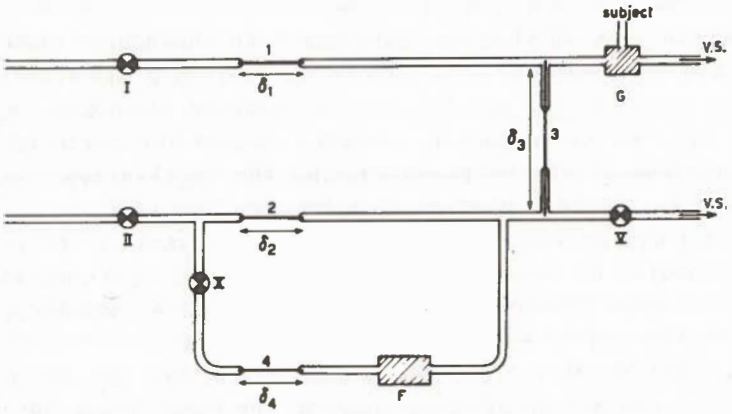


Figure 1a
Diagram of the apparatus. The capillaries are marked by 1, ... 4; the pressures by δ_1 .... δ_4 and the stopcocks by I, II, V and X. F is the vessel containing the odorant, G the injection apparatus and V.S. the ventilating system.

the observer. The main stream receives an adjustable amount of odorous nitrogen from the second line, which finally receives a current of nitrogen, saturated with odorous molecules from the third line. To prevent oxydation of the odorant, nitrogen is used in the second and third line.

The stopcocks I, II and X control the rate of flow of the air or nitrogen passing the three lines.

The rate of flow of the odorous nitrogen passing capillary 3 is controlled by means of stopcock V, because stopcock V controls pressure δ_3. Normally, the main part of the current of odorous nitrogen in the second line is removed by way of the ventilating system V.S., only a small part is supplied to the main line.

The concentration of the odorant in the third line can be determined from the vapour pressure of the odorant through which the stream of nitrogen is led (F) if the nitrogen is saturated with odorous molecules. When the stream of odorous nitrogen enters the second line, the saturated nitrogen is diluted by the pure nitrogen passing capillary 2. The concentration of the odorant in the second line can be determined from the rates of flow in capillaries 2 and 4.

A small part of the odorous nitrogen in the second line is sent to the main line by way of capillary 3. When the rates of flow in this capillary and the main line are known, the concen-

tration of the odorant in the main line can be calculated from the concentration in the second line.

When the rate of flow in capillary 3 is changed by means of stopcock V, the rates of flow in the capillaries 2 and 4 are also slightly changed. The dilution of the odorous substance in the second line is not affected, however, since the ratio of the rates of flow of the nitrogen passing the capillaries 2 and 4 only depends on the dimensions of these capillaries.

The air can be supplied to the subject by means of the injection apparatus G. When the air is not injected, the current of air in the main line normally passes the injection apparatus and is removed by way of the ventilating system V.S.

The capillaries 1, 2, 3 and 4 were calibrated one by one by collecting the air or nitrogen passing the capillaries. For the small capillaries 2, 3 and 4 only one calibration for one pressure was required because the rate of flow was proportional to pressure (Poisseulle). Capillary 1 was calibrated for the whole range of pressures used during the experiments; since the rate of flow in this capillary is much larger than in the other capillaries the pressure increases faster than proportional to the rate of flow. So the rates of flow of the air or nitrogen passing the capillaries can be determined from the pressures δ_1, ..., δ_4 and the data obtained from the calibration of the capillaries.

If the threshold experiments are started, one has first to find the rough magnitude of the threshold. Normally, one has to try different capillaries 3 before the required range of concentrations is found. For this purpose, three different capillaries between the second and the main line are mounted in our apparatus simultaneously. The capillaries can be used separately (see fig. 1b).

Once a suitable capillary has been found, the concentration in the main line is controlled by the stopcocks V and X (pressures δ_3 and δ_4). Normally, one has a sufficient control of the number of odorous molecules in the main line by changing the pressure δ_3 only (with the aid of stopcock V).

In figure 1b the apparatus is given in more detail. Instead of the open manometers used in our apparatus, differential manometers can also be used for determining the rate of flow of the air or nitrogen passing the capillaries. So the pressure δ_1, given by a differential manometer is equal to P_1-P_5, δ_2 is equal to P_2-P_3, etc. The manometers P_5 and P_3 are filled with water, the others with mercury. The manometer P_3 has to be attached to the appara-

tus on a place where no odorous molecules pass the second line, because otherwise odorous material can dissolve in the water used in the manometer. For the same reason no grease is used for stopcocks IV, VI and VIII.

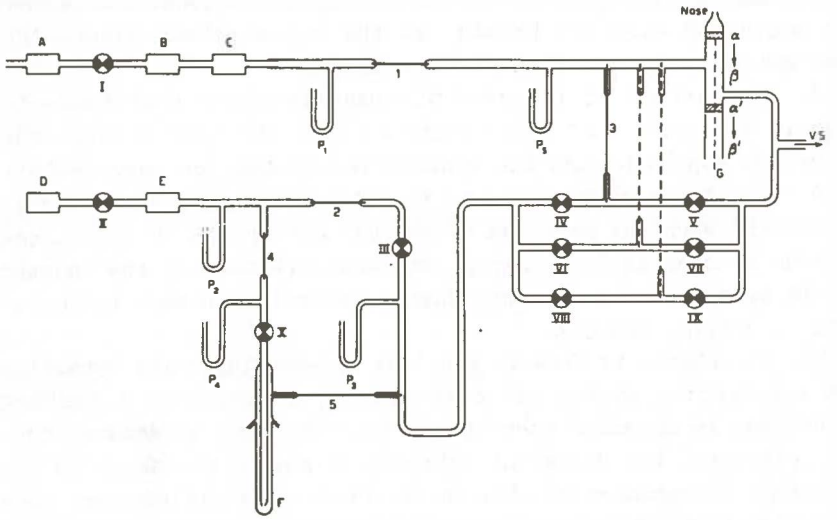


Figure 1b
Diagram of the equipment. The capillaries are marked by 1, 2, ... 5; the stopcocks by I, II, ... X and the pressures by P_1, P_2, ... P_5. Normally the positions of the disks are α and α', in working position β and β'. A: compressor, B: washing device, C: equipment for obtaining air with a fixed temperature and humidity, D: nitrogen flask, E: purification of the nitrogen, F: vessel containing the odorant, G: the injection apparatus, V.S.: ventilating system.

The air current in the main line is supplied by a rotating compressor (A). After leaving the compressor, the air is washed (B) and brought to a fixed humidity (32%) and temperature (17 °C) (C). To obtain a constant humidity, the air is first saturated at room temperature with water vapour, then cooled down to 0°C and heated to the desired temperature. The maximum capacity of the compressor is about 900 cc per second.

The air, supplied by the compressor, normally passes the injection apparatus and escapes into the open air by way of a ventilating system. The injection apparatus consists of a standing cylinder, which has two movable pistons mounted on one bar (see fig. 1*b*). The air normally passes from the inlet tube into the outlet tube V.S., because the pistons are in position α (fig. 1*b*). A relay can shift the pistons to position β. For the position α of the pistons the air passing the injection apparatus is removed

by means of the ventilating system; during an injection the position of the pistons is given by β. The air is then forced through the nosepiece.

Before injection was started, the subject inserted the nosepiece into the nostril. During injection, the subject has opened his mouth and hold his breath; so the injected air passes the nose and then escapes by way of the mouth.

As the current of injected air experiences a resistance to flow in the nose, it is necessary to give the line between the injection apparatus and the ventilating system the same resistance. When these resistances to flow are not equal, the pressure P_5 changes when the position of the pistons in the injection apparatus is changed from α to β. The concentration of the odorant in the main line changes then during injection because the pressure $\delta_3 = P_3 - P_5$ changes.

The resistance to flow in the line between injection apparatus and ventilating system is controlled by a stopcock; a correct adjustment is obtained when the pressure P_5 does not change during injection. For different subjects it may be necessary to use different resistances to flow in the line; even for the same subject this resistance may alter sometimes from day to day.

Of course it is also possible to use the injection apparatus for smell experiments when normal breathing is required. The injection apparatus may be switched on during a few seconds, in this interval of time the subject takes an inspiration in the current of odorous air which leaves the injection apparatus through a hole with a diameter of 11.6 cm.

The current of nitrogen in the second line is supplied by a nitrogen flask (D); a rate of flow up to 10 cc per second is used. The nitrogen current is purified by the use of coal and silicagel as adsorbents, and a cooling system with liquid air as cooling material (E). A small part of this nitrogen, up to 1 cc per second, is sent through the odorous material in vessel F. It is also possible to send the whole nitrogen current through the vessel by closing stopcock III. The flow through vessel F has to be small because the nitrogen should be saturated with the odorant. To get a constant concentration of the odorant in the third line the vessel is kept at a constant temperature. For substances with very low olfactory thresholds, e.g. mercaptans, there are two possibilities. The vapour pressure may be lowered by cooling the vessel down to -72°C or more, or very small capillaries may be used to get a small amount of odorous material in the odorous

line. Both procedures have been used by us. It is better to cool the odorous substance, since accidental escape from the vessel will not spoil the air in the room so badly.

The capillary 5 in our apparatus, which is a wide one, only served to bring the small flow of nitrogen passing the third line into the centre of the second line.

In order to calculate the number of molecules used for the stimulation it is necessary for the air, passing the odorous substance, to be saturated with odorous molecules. With the use of the vapour pressure data collected by Jordan (9), it is possible to calculate the vapour pressure of various organic substances used for our experiments. For meta-xylene a value of 1.75 mm Hg at 0°C was determined by extrapolation.

To check the saturation with odorous molecules we determined the loss of weight of the meta-xylene. This loss appeared to be 0.740 grams when an air current of 2.4 cc per second was sent through the odorous substance during 7 hours. Assuming saturation, one may expect a loss of 0.724 grams in this case (calculated from the vapour pressure of 1.75 mm Hg.), which is in good agreement with the experimental results. The slight difference may result from experimental inaccuracies. So we may conclude that the air is saturated. The rate of flow of 2.4 cc per second through the vessel is large, compared to the rates of flow normally used. The air is then certainly saturated at the normal rates of flow.

The entire apparatus, except the valve, has been made of glass. We have used two valves, one made from perspex and one from nickelplated brass. The perspex valve, however, produced an odour caused by the friction between the moving disks and the standing cylinder. The nosepiece has been made from perspex and fills the entrance of the nostril entirely. It penetrates about 1.5 cm into the nostril. During stimulation the air leaves the nosepiece through a hole with dimensions of 12 × 3 mm.

The duration of each injection can be adjusted to any time desired between 0.08 and 2.0 seconds. For this purpose an univibrator is used, which can be triggered by the subject by means of a switch.

A suitable point of the univibrator circuit is connected with the grid of an output tube; without an input-signal from the univibrator this tube is cut off, otherwise is conducts. When the output tube conducts, the signal relay 1 is put in working position. A current of 2 ampères is then supplied to relay 2, which

changes the position of the pistons of the apparatus used for the injection of the odorous air.

It is necessary to check the duration of each injection because the mechanical properties of the valve (friction) may vary a little. This is done by using a metal strip, which makes contact with another when piston α is in the centre of the aperture of the inlettube. Of course this contact is interrupted when the valve reverts to its former position. When contact is made, the pulses, produced by an oscillator are counted by a scaler. The injection time is the counted number of pulses, multiplied by the average duration of one pulse.

To reduce losses during stimulation, it is important to have the nosepiece filled with odorous air. For this purpose a small amount of odorous air leaks continuously along piston α. It is clear that then losses of odorous air during stimulation only occurs in the nasal cavity itself.

Of course this leak should be very small. If one uses a nose piece with a volume of a few cc and gives one stimulation a minute, a leak of 1/10 cc per second is sufficient to fill the nose piece with odorous air. This leak should not be made much larger, because otherwise already before the normal injection begins, some odorous air will leak into the nasal cavity when the subject inserts the nosepiece into the nostril. The very small space between piston α and the standing cylinder, which is always present since the friction would otherwise be too large, is sufficient for this small leak.

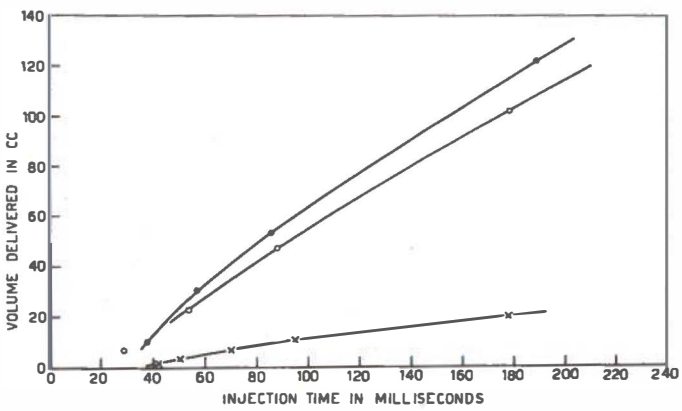


Figure 2
The volume delivered by the injection apparatus for various injection times. Three different rates of flow were used.

One may expect that the volume, delivered by the apparatus is proportional to the injection time. We checked this by collecting the delivered air. The results have been plotted in fig. 2. The volume delivered appeared to be proportional to the injection time above 0.08 second; below this time the delivered volume drops faster (fig. 2). So in general there is no point in using injection times shorter than 80 milliseconds with our apparatus. The deviation from linearity can be understood as follows: As stated before the injection time is the interval between two subsequent passings of the middle of the inlet tube. At very small injection times, the disk is already moving back before the whole aperture of the inlet tube has been passed. At somewhat larger times, the disk just passes the opening. Since the velocity in the neighbourhood of the reversing point of the disk is small, the aperture is partly blocked during part of the injection time. The influence of this blocking can be seen in fig. 2.

Some odorants show the property to adhere strongly to glass surfaces, e.g. 1:4-xylen-2-ol. For these substances it is necessary to get an equilibrium between the number of molecules which adhere to the glass surfaces in the odorous line (and capillary 3) and the number of odorous molecules released by these surfaces. This is obtained by sending a nitrogen current for a few hours through the odorous line and the vessel F; the rate of this current must be about the same as the rate used during the experiments. Part of this current goes through capillary 3. For the substance 1:4-xylen-2-ol the number of molecules delivered at the injection apparatus increased during the first hours a factor 10, when the adjustment of the apparatus was not changed, before a constant number was reached. m-Xylene, e.g. reached a constant threshold immediately. The adsorption in the odorous line appeared to be smaller for larger rates of flow in this line.

To prevent adsorption as much as possible, the length and diameter of the main line between capillary 3 and the nose piece is kept small. The length of this part is about 25 cm, the inner diameter 0.5 cm. It is not possible to use much thinner tubes, because the pressure P_5 at the end of capillary 3 would become too large. (The difference P_3-P_5 determines the flow passing capillary 3). In our case we have to apply a correction to the read pressure P_5, because the attachment of the manometer does not coincide with the end of capillary 3. The correction is different for the 3 capillaries mounted in the apparatus.

The adsorption in the main line is small for most of the odor-

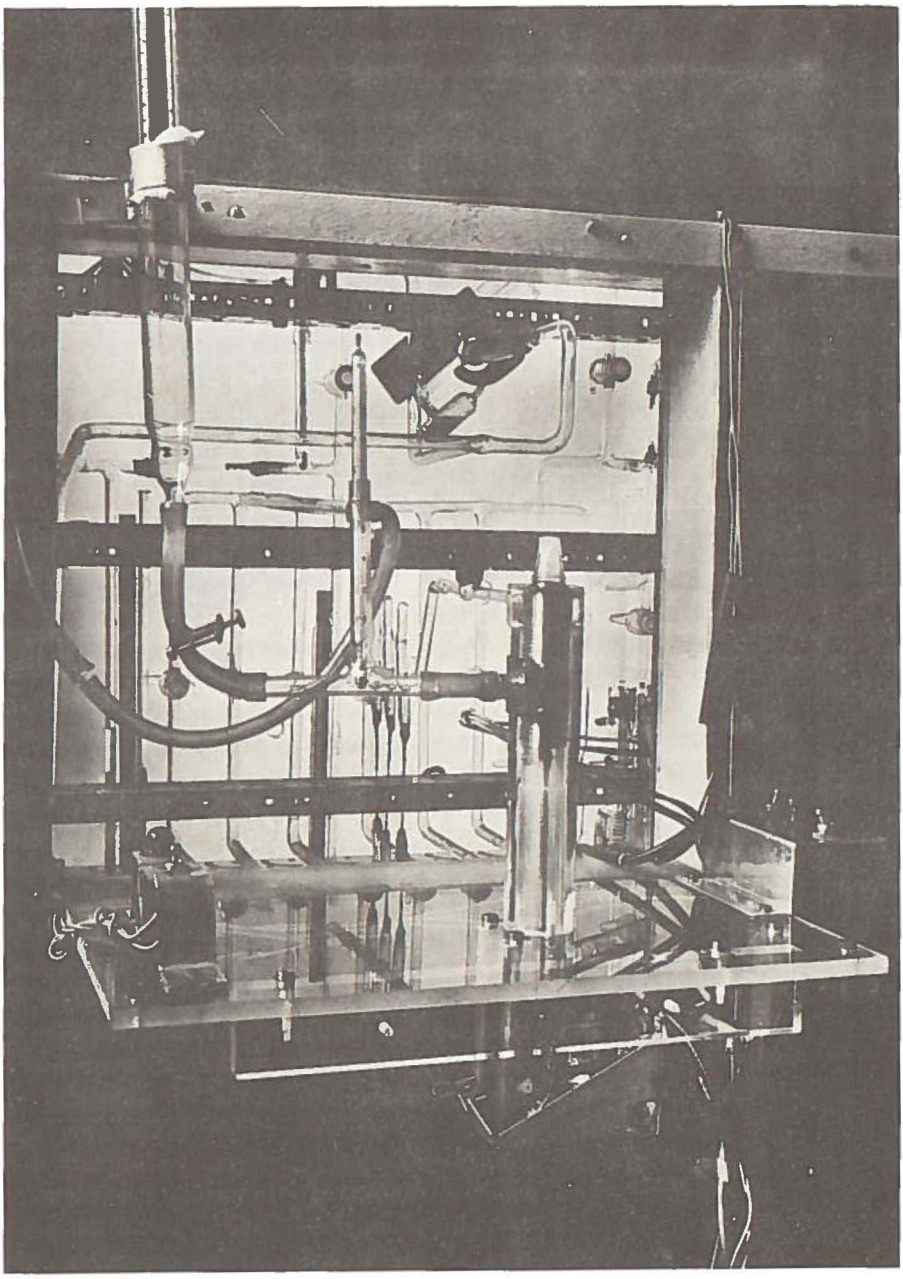


Figure 3
The injection apparatus.

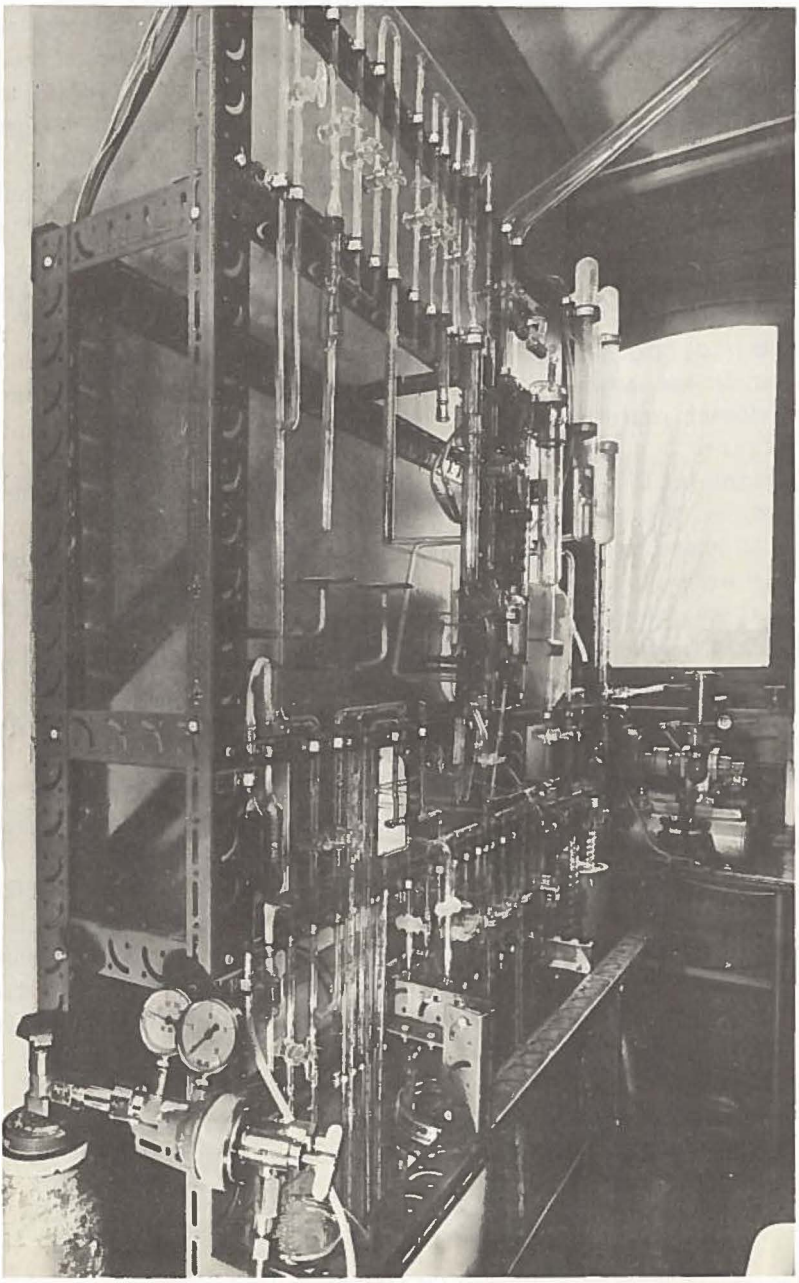


Figure 4
The equipment used for the adjustment
of the stimulus.

ous substances used. The following experiment was performed with a rate of flow of 300 cc per second. The pressure P_3 was adjusted in such a way that the concentration of the d-octanol in the main line was 30 times the threshold concentration. When the pressure P_3 was suddenly set back to zero, the smell of the odorant disappeared within 5 seconds. At 10 times the threshold concentration the smell disappeared within 3 seconds. For m-xylene the times were even shorter.

Our apparatus, which has been designed independently of existing equipments, has some resemblance to those of Gundlach and Kenway (10) and Neuhaus (11). With the apparatus of Gundlach and Kenway it was not possible, however, to vary the concentration of the odorant rapidly.

Figures 3 and 4 show two photographs of the apparatus. The injection apparatus is shown in figure 3. Part of the apparatus needed for the adjustment of the stimulus can be seen through the perspex plate in a hole in the cabin. The injection apparatus is mainly made of perspex, but the sliding pistons and the inner part of the apparatus are made from nickel plated brass. At the left side of the apparatus the connection to the ventilating system can be seen. The nose piece is placed on the apparatus. At the back, the main line is connected to the apparatus, the three capillaries for supplying odorous material in this main line can easily be seen. The switches are for signalling results and for starting the injection. The relay is placed below the perspex table.

The apparatus used for the adjustment of the stimulus is shown in figure 4. The equipment shown at the upper part of the left side of the photograph is not used for the experiments discussed in this thesis. In the foreground the nitrogen flask, at the back the compressor. Close to the top at the right side the washing device is placed.

Chapter II

THE INFLUENCE OF THE STIMULUS DURATION UPON THE THRESHOLD

2.1. *Introduction*

In this chapter some experiments concerning the influence of the stimulus duration upon the threshold of the human sense of smell are discussed.

For the sense of sight and hearing the relation between duration of stimulus and stimulus intensity has been studied extensively. Below a certain critical time the product of light- or sound-intensity and stimulus duration proved to be constant for threshold stimulation. So a constant amount of energy is required for the production of a constant photochemical effect. If the stimulus duration is larger than the critical duration, the threshold intensity remains independent of time. When the stimulus is restricted to small areas of the sense organ the changeover is rather abrupt; when larger areas are used the changeover is smooth. For the olfactory sense this relation has not yet been studied, probably in consequence of the fact that the determination of olfactory thresholds is a complicated matter.

When using the "blast injection technique", discussed in chapter I, for the determination of olfactory thresholds, time, volume and rate of flow of the injected air are exactly known. However, it is emphasized in this chapter that the relative losses before the air reaches the epithelium are dependent on the injected volume. A correction of the measured threshold is necessary, therefore. If this injected volume, compared with the volume of the nasal cavity, is not large, the correction is important. This will be discussed in 2.3.

In our experiments the intensity-duration function has been determined for three compounds, secondary butyl mercaptan, m-xylene and o-nitrophenol. The stimulation times used range from 0.08 to 2.0 seconds.

2.2. *Experimental procedure*

Analogous to other sense organs, there is for the sense of smell no sudden transition between smelling nothing and smelling an odorous substance, when the number of odorous molecules striking the sense organ increases. For a given concentration the subject may smell the substance for instance 2 out of each 10 times, for a somewhat larger concentration this may be 8 out of each 10 times. If one determines the percentage of positive responses for various amounts of odorous substance, one gets a "frequency of smelling curve". The olfactory threshold is defined here as the number of injected molecules required for a frequency of smelling of 50 percent.

To determine a threshold about 40 stimuli, spread over 2 or 3 different concentrations of odorous substance are presented to the subject. These different quantities (concentrations) are obtained by changing pressure P_3 with stopcock V (see 1.2, fig. 1b). The subject receives about one stimulus a minute.

The whole equipment, except the valve is placed in a room entirely separated from the subject. There is a circuit for necessary signalling between the subject and this room. Temperature is kept constant in the subject's room, while both rooms can be ventilated.

There proved to be fluctuations in the sensitivity of the subject from day to day. These fluctuations are small, if the subject does not smoke at all and does not eat and drink substances with a strong odour. m-Xylene, e.g., was used for five days from 11-2-1957 till 19-2-1957 and on 1-3-1957. On these days the thresholds for the same injection time and rate of flow were 7.6×10^{13}; 6.1×10^{13}; 2.8×10^{13}; 8.0×10^{13}; 5.4×10^{13} and 2.8×10^{13} molecules respectively. The deviation from the average value does not exceed a factor of 2. The same experience is obtained with other odorous substances.

If there are determinations of thresholds of a given substance on different days, it is necessary to take these fluctuations into account. During experimentation the threshold belonging to one fixed injection time and rate of flow is determined daily. The average value of these thresholds is calculated. If the determined threshold on a certain day proves to be a factor p higher than the average value, all results of this day are divided by this factor p. If corrected for the daily variations of sensitivity, the thresholds with the same injection time and rate of flow have only a small spread (see fig. 6).

For checking purposes stimuli without odorous substance are also given. Normally the number of positive responses for pure air is lower than 5 percent, the threshold determinations with a higher response have been omitted. The subject should not be uncertain in his decisions and there should be no shifting of the olfactory threshold during the experiments in order to get intensity-duration curves with a small spread. It is especially owing to the former condition that good results are obtained only a few days a week, mostly in the morning. The shifting of the olfactory threshold will be caused by partial blocking of the epithelium by some mobile nasal mucus. The stimulation is monorhinic, all the results have been obtained with the author as subject.

2.3. *Corrections*

First of all it is necessary to consider the differences in the losses for the various volumes of injected air. The losses are the following:

1. Losses due to adsorption before the epithelium is reached. To determine the influence of these losses the number of injected molecules necessary for threshold stimulation at an injection time of 0.55 second and for different rates of flow was determined. The odorant used was m-xylene. When using rates of flow with a range of 100 cc up to 200 cc per second the liminal number of injected molecules remained approximately constant. This means that in spite of the fact that the concentration for a rate of flow of 100 cc per second is twice as large as for 200 cc per second, the threshold remains approximately constant. As will be discussed in 3.2 the fraction of odorous molecules lost by absorption is different for various rates of flow. However, the fraction of the molecules striking the walls of the olfactory slit varies also with the rate of flow and the result of both effects in this case is apparently that the threshold remains approximately constant.
2. The losses caused by the fact that when the injection stops, the foremost part of the nasal cavity has been filled with odorous air. The bulk of this air does not reach the epithelium. Expressed in cc this loss will be the same for all volumes injected, but it has relatively the greatest influence when small volumes are used. When working with a fixed rate of flow, the correction is largest when small times are used.

An estimate of this loss can be made as follows:

If an injection time of 0.2 second is used, the number of injected molecules of m-xylene necessary for threshold stimulation decreases with 7 percent, when the rate of flow is changed from 100 to 200 cc per second. This decrease is 16 percent for an injection time of 0.1 second. The differences mentioned here must be partly due to the volume lost in the nasal cavity. Suppose this loss to be A cc, then the fractions reaching the epithelium are proportional to 10 – A and 20 – A for an injection time of 0.1 second and rates of flow of 100 and 200 cc per second respectively, if the fraction of the number of injected molecules reaching the epithelium is the same. From our observations we get the equation:

$$\frac{10 - A}{10} : \frac{20 - A}{20} = 6 : 7 ,$$

which gives for A: 2.5 cc.

As will be discussed in chapter III, the fraction of the number of molecules reaching the epithelium is normally not the same for different rates of flow. Only in this special case it is approximately the same. If one assumes that the whole difference of 7 percent at 200 ms is caused by differences in absorption in the mucous membrane and efficiency in the olfactory slit, one gets for the volume A: 1.5 cc (calculated from the results for an injection time of 0.1 second). So a value of 2.0 cc will not differ too much from the real value; however, it should be borne in mind that it is merely an approximation.

The value of 2.0 cc is a reasonable one compared with the volume of the foremost part of the nasal cavity, through which the odorous air has to pass. Using 100 cc per second, the corrections of the injected number of molecules necessary for threshold stimulation for times longer than half a second, are negligible. The molecules injected at 0.1, 0.2 and 0.5 second and a rate of flow of 100 cc per second are lost for 20, 10 and 4 percent respectively, in consequence of the lost volume in the nasal cavity. When a flow of air of 200 cc per second is used, the losses are 10, 5 and 2 percent for injection times of 0.1, 0.2 and 0.5 second respectively. At 0.5 second the difference in losses due to the volume lost in the nasal cavity is only 2 percent for a rate of flow of 100 and 200 cc per second, which is in good agreement with the experimental results, showing that for this injection time the differences due to absorption and lost volume in the thresholds measured are hardly detectable. Of course this value of 2.0 cc will greatly depend on the subject used for the experi-

ments and will even vary for the same person from day to day. These variations for the same person are due to the fact that small mucous particles strongly influence the losses by deflecting the flow of air. The value of 2.0 cc must be considered as an average value, therefore.

3. The real stimulation time is also shorter than the injection time, because the first part of the stimulation occurs with clean air, driven up by the odorous air. Using a rate of flow of 100 cc per second and assuming a volume of 2.0 cc for the clean air, the injection times must be decreased by 0.02 second to find the real stimulation times. Real stimulation time means the duration of the stimulus supplied to the epithelium.

When the injection pulse stops, the odorous molecules in the olfactory slit diffuse to the walls of the slit. The diffusion time necessary for a 10 percent decrease of the number of molecules in the olfactory slit is of the order of 1 or 2 milliseconds (chapter III). For threshold measurements a 10 percent decrease of the number of molecules always gives a large decrease of the percentage of positive responses (3.4). So when the injection pulse stops the stimulus comes below the threshold value within a few milliseconds.

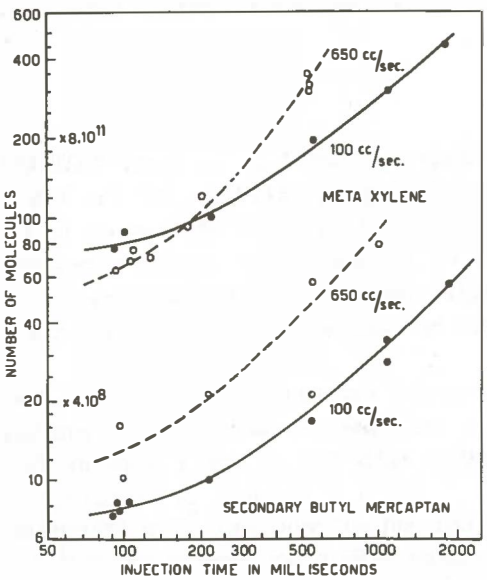


Figure 5
The absolute threshold, expressed in number of molecules injected, as a function of the injection time. Two different rates of flow were used. No corrections were applied.

4. Figure 5 represents the results of threshold measurements for two different rates of flow, 650 and 100 cc per second. No corrections have been applied. The curves for 650 cc per second have a straighter course than those for 100 cc per second. It should not be concluded however, that this is the result of lower losses at small volumes, owing to the fact that the curve is a result of a competition between an odorous sensation and another sensation. There appears to be a strong sensation due to the pure air alone, if one uses a rate of flow larger than 200 – 300 cc per second. This results in an increasing threshold for smelling. The sensation may arise from a drying effect of the injected air on the olfactory receptors or from the cutaneous receptors of the nostrils. At times of 0.1 and 0.2 second the sensation is not so strong as at larger times. At short times one has the impression that this sensation and the odorous sensation are more or less separated, the odorous sensation arriving later. Rates of flow larger than 200 – 300 cc per second should not be used, therefore. For meta xylene the curves intersect; for secondary butyl mercaptan, however, no intersection occurs. This will be caused by the fact that for m-xylene the sensation produced by the fast air current does not dominate the smell sensation to such a large extent as this is the case for secondary butyl mercaptan.

2.4. *Results*

Looking at figures 6 and 7 it is clear that for the olfactory sense the same relation is valid as for the eye and the ear. At the right side the inclination of the curves in figure 6 indicate that the number of molecules per second necessary for threshold stimulation, approaches a constant value above a given time. At the left we have an approach to the region of a constant number of molecules.

The dotted curves represented in figures 6 and 7 have been deduced from the experimental curves by decreasing the number of injected molecules with 20, 10 and 4 percent for the injection times of 0.1, 0.2 and 0.5 seconds respectively, when a rate of flow of 100 cc per second was used. The injection times are decreased in this case with 0.02 seconds to find the real stimulation times. For the upper curve in figure 6, which has been determined for a rate of flow of 270 cc per second, the correction for the dead volume is 8 percent for an injection time of 100

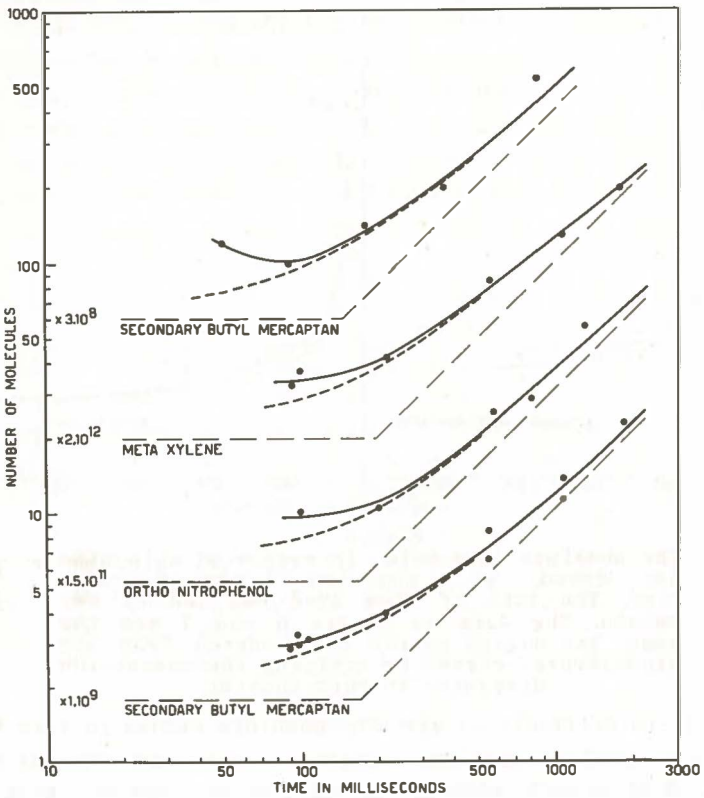


Figure 6
The absolute threshold, expressed in number of molecules injected, as a function of the injection time. Two different rates of flow were used, for the upper curve 270 cc per second, for the other curves 100 cc per second. The dotted curves are deduced from the experimental curves, the correction applied is discussed in this chapter.

milliseconds. For an injection time of 50 milliseconds the correction is much larger because the volume delivered by the apparatus is not linear with injection time (see 1.2, figure 2). The total correction is about 40 percent in this case. For the correction of the dead volume see 2.3. This corrected number of molecules is not the number reaching the olfactory receptors. The fraction reaching the epithelium is about 4 percent (see chapter III). As pointed out already in 2.2, the thresholds have been corrected for the daily variations of sensitivity.

The critical times are about 0.16 second for secondary butyl mercaptan, 0.20 second for m-xylene and 0.17 second for o-nitro-

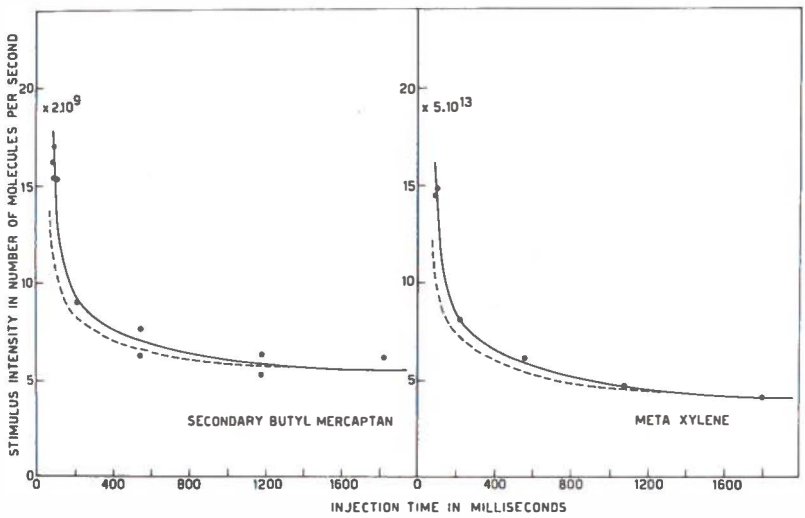


Figure 7
The absolute threshold, in number of molecules per second, as a function of the injection time. The rate of flow used was 100 cc per second. The data in figure 6 and 7 are the same. The dotted curves are deduced from the experimental curves by applying the correction discussed in this chapter.

phenol. It is difficult to give the possible errors in this time. We can only conclude that the critical time of the sense of smell is about 0.18 second, whereas the differences for the used substances are probably not significant. This time is in good agreement with the critical times of the eye and the ear, which are 0.10 and 0.20 seconds respectively.

In figure 7 the relation between the number of molecules per second for threshold stimulation and the stimulus duration is plotted. The underlying data are the same as used in fig. 6. It is only the manner of representation which is different. The number of molecules per second corresponds with the light intensity in the case of the eye. This number divided by the rate of flow also gives the concentration of the odorous substance in the injected air. For threshold stimulation at small times it is necessary to increase the concentration.

For the calculation of the total number of injected molecules we need the vapour pressure of the odorant at 0°C. The following vapour pressures have been used: o-nitrophenol: 5.10^{-2} mm Hg, m-xylene: 1.75 mm Hg and secondary butyl mercaptan: 23 mm Hg, all at 0°C.

The lowest numbers of injected molecules necessary for threshold stimulation are, in our case, 2.10^{9} for secondary butyl mercaptan, 4.10^{13} for m-xylene and 8.10^{11} for o-nitrophenol.

Lastly a discussion of the critical duration. Irrespective of the results with other sense organs we can hardly expect a very short critical duration for the sense of smell because of the fact that the molecules reaching the epithelium need a certain period of time to diffuse to the sense cells. According to Kolmer (12), the epithelium is covered by a thin waterlayer. Then a diffusion time of about 20 milliseconds is required for a molecule to diffuse to the sense cells (see 3.4). Perhaps there is also diffusion through other layers, before the sense cell is activated. So a very short injection of molecules in the nose probably always has an action of about 100 milliseconds.

Chapter III

THE ABSOLUTE SENSITIVITY OF THE SENSE OF SMELL IN MAN

3.1. *Introduction*

For checking possible olfactory theories it is of importance to know whether a response of the receptors is caused by "large" amounts of odorous substance, so that the rules of chemistry concerning concentrations etc. can be applied, or whether a receptor is already activated by one molecule. For a theory of olfaction it is also important to know the efficiency of the olfactory process and the number of sense cells which have to respond simultaneously for a sensation to be perceived.

The experiments discussed in this chapter have been performed to get some information on above problems. The first experimental problem is the determination of the number of molecules striking the olfactory epithelium. This number is difficult to determine because only the number of molecules entering the nose is known. To solve the problem, it is necessary to determine the fraction of the air passing the olfactory slit. For the determination of this fraction some experiments have been made with a model of the lumen of the nasal cavity. The results are given in 3.2.

When the fraction of the air passing the olfactory slit is known it is possible to calculate the number of molecules striking the epithelium. For this calculation it is necessary to take into account the absorption of odorous molecules in the mucous membrane before the olfactory slit is reached and the diffusion of the odorous molecules in the slit, which determines the fraction of the molecules contacting the walls of the slit. These calculations are given in 3.3.

When the number of molecules striking the olfactory epithelium has been determined, it is possible to calculate the minimum number of molecules by which a receptor is activated. On the *assumption* that the "frequency of smelling" curves are caused by statistical fluctuations in the number of molecules striking the olfactory epithelium, it is possible to give an estimate of the

number of receptors which have to act together simultaneously for a sensation to be perceived. The discussions of this problem are given in 3.4.

In 3.4 a discussion of the efficiency of the olfactory process is also given.

3.2. *The fraction of the air passing the olfactory slit*

The fraction of the air passing the olfactory slit depends on the method of injection or inhalation used.

One method consists of normal inhalation of air containing an odorant, the other consists of injection of a given volume of odorous air into the nose. Injection in one nostril is called monorhinic, in two simultaneously dirhinic.

First we shall discuss normal breathing. This method has the disadvantage that for subsequent inspirations volume and rate of flow of the inhaled air may vary. However, with well-trained subjects, the influence of these variations upon threshold determinations appears to be rather small.

The structure of the nose is of fundamental importance in solving the problem. It is a well-known fact that the two nasal cavities are separated by the nasal septum. In man there are three conchae in each nasal chamber. The contours of the walls of the nasal fossae in living beings are such that both the upper and the lower nasal passages are mere slits, nowhere wider than 1-2 mm. The capacity of the nasal chambers is always larger in cadavers than in living beings; this is caused by shrinking of the conchae.

The epithelium of the olfactory region is situated in the upper part of the nasal chamber. The olfactory area extends from the top of the cavity to about 1 cm below the top along the lateral wall and the septum. The area of the olfactory epithelium is about 2.5 cm^2.

According to Proetz (13) the general pattern of the normal air currents is determined by three structural elements; the direction of the anterior naris, the essential configuration of the nasal chamber and the relative sizes of anterior and posterior nares. The narrow passage joining the vestibule to the proper nasal chamber is the most constricted point of the respiratory tract, the inspired air is directed here almost vertically to the top of the chamber. This vertical air current presently spreads

in a wide and slanting curve towards the choana all but avoiding the inferior part of the chamber.

Published articles set forth almost any type of pathway conceivable within the anatomical restrictions of the nose. Proetz has given an extensive survey of these articles. According to him, the disagreement is the result of the fact that the normal nose is characterized by slit-like passages, but that upon examination of these passages with a nasal speculum or a pharyngoscope the passages are widened. With the aid of a model, Proetz comes to the conclusion that the air currents are shifted to the lower half of the nasal chamber only when the upper passages become so narrow that they have to be considered abnormal. So under normal conditions the air certainly passes the olfactory region.

Various experimenters, using models of cadavers, state that for normal breathing there are no eddy currents in the nasal cavity. As discussed above, the nasal cavities of living beings differ from those of cadavers. A better argument for the lack of eddy currents in the nasal cavity is that an odorous substance is only smelled for normal inspiration when placed in specific spots before the nose. (Zwaardemaker (14)). If eddy currents should occur extensively the odorant would be smelled wherever the source would be placed in the respiration flow.

The lumen of the human nose can be represented by the model of fig. 8. For calculations or experiments on the air currents this model is very suitable, but it should be borne in mind that great individual differences are possible. Even for the same person the lumen of the nose can vary considerably by swelling or shrinking of the conchae by control of their blood supply. Essential for

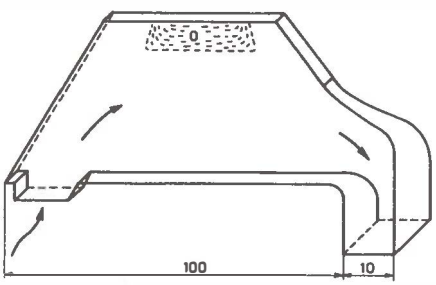


Figure 8
Schematical representation of the lumen of the human nose. The olfactory epithelium is situated in the top of the cavity, the area 0 is about 2.5 cm^2.

the model are the trapezoid shape and the small distance between the septum and the lateral wall.

To determine the fraction of the air passing the olfactory slit some experiments with the model have been conducted. Water was sucked through the model with different rates of flow. To get the same streamlines as in the air the rates of flow in air and water have to be proportional to the kinematic viscosities. The viscosities for water and air are 0.010 and 0.00018 Poise respectively. The ratio of the densities is 1:830. So the ratio of the kinematic viscosities is 1:15. The rate of flow of water has to be 15 times smaller than for air so as to get the same streamlines. For normal inspiration, the volume of inhaled air is about 250 cc for one nose side, while the inspiration time is about 1 second. A corresponding rate of flow for the water is 16.5 cc per second.

During experiments a suspension of small aluminium particles is brought into the water at the entrance of the model. The model was made of perspex in order to see the streamlines. In fig. 9 the streamlines for normal breathing are given. If the concentration of the aluminium particles is not too large the spurs of the particles can be photographed. From the length of the spurs in the olfactory slit and the time of exposure it is possible to calculate the rate of flow of the water in the slit.

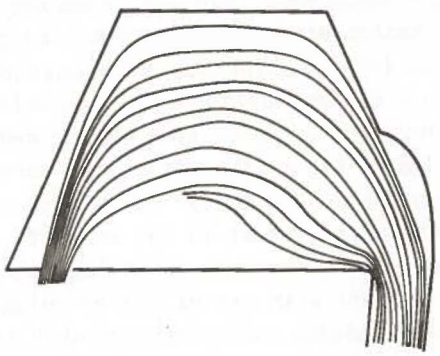


Figure 9
Schematical representation of the streamlines in the model for normal breathing.

There appears to be some scattering in the results since the length of the spurs depends on the distance of the aluminium par-

ticles from the wall. Indeed exact results are not important because individual differences of the structure of the nose are large. The following results were obtained:

1. No air currents pass the inferior parts at normal breathing (250 cc per sec per nasal chamber). The air passes through the part of the cavity lying between the top and a point which is 1.5 cm above the base. So only a 2/3 fraction of the cavity is used.
2. For normal inspiration the fraction of the inspired air passing the olfactory slit is at least 5 and at most 10 percent.
3. At larger rates of flow this fraction becomes larger because the air current is directed towards the top of the chamber. According to our experiments percentages larger than 20 percent are not to be expected.

3.3. *The fraction of the molecules inhaled or injected striking the olfactory epithelium*

In order to determine the fraction of the molecules striking the olfactory epithelium it is necessary to know what happens to the odorous molecules there. According to Moncrief (15) it is a process of adsorption; a dynamic equilibrium adjusts itself, so that at each moment molecules arrive at the epithelium and an equal number is taken off. However, such an interpretation is contradictory to the experience that the sensation of smell stops suddenly when inspiration stops. According to the assumption of a dynamic equilibrium in the olfactory slit molecules are still striking and leaving the epithelium. The sensation cannot stop all at once, therefore. A very quick stop of the sensation is only possible if the width of the slit is small, because otherwise the diffusion time of the molecules is too large, and if moreover the main part of the molecules is lost in the specific receptormechanism or other parts of the olfactory epithelium at the first time the molecules make contact with the olfactory epithelium.

If the air current passing the olfactory slit is stopped, the odorous molecules remaining in the slit will diffuse to the walls. We shall assume now that each molecule contacting the wall has no opportunity to leave the epithelium. Assuming a width d of the olfactory slit and a coefficient of diffusion D of the odorant, the number of molecules N remaining in the slit after a certain time t is in fair approximation given by

$$N = N_0 \, e^{-D\pi^2 t/d^2}, \qquad (1)$$

where N_0 is the number of molecules in the olfactory slit when inspiration stops. According to the data given in "Critical Tables" (16) the coefficients of diffusion range between 0.04 and 0.12 cm^2/second for a large number of odorants. In the following discussion we shall except a value of 0.08 cm^2/second. For this value of the coefficient of diffusion and a width of the olfactory slit of 1 mm, the number N of remaining molecules in the slit after the time t is represented by

$$N = N_0 e^{-80t} . \qquad (2)$$

When a person is breathing in an environment in which the concentration of an odorant is 10 times the threshold concentration, the stimulus does not come below the threshold before the number of molecules in the olfactory slit is one tenth of the number of molecules in the slit at normal inspiration. According to formula 2, this is the case after 30 milliseconds when inspiration has ceased. Assuming a width of 2 mm for the slit, this time is 120 milliseconds. So the number of molecules striking the epithelium will very soon drop below the threshold value, according to our assumption that each molecule contacting the wall has no opportunity to leave the olfactory epithelium. If half of the molecules are absorbed the first time they make contact, the times will be nearly twice as long.

Now the following experiment was made: During some time, not too long to prevent adaptation, an air current was sent into the nose. The concentration of the odorant in this air current was 10 times the threshold concentration. During a short interval the air current was interrupted. The interruption time was registered. The purpose of the experiment was the determination of the shortest possible interruption time by which the smell sensations are separately perceived. The experiments were made with two subjects. Both reported that it was difficult to determine the demanded time exactly. So the recorded times are approximations. On the average this time appeared to be about 180 milliseconds for d-octanol.

It is very probable, however, that the sensation does not stop at the moment when the stimulus comes below the threshold, but lasts somewhat longer. So the time of 180 milliseconds is the result of two effects; first because the molecules need a certain time to diffuse to the walls (and to the sense cells) and second because the sensation probably lasts somewhat longer than the

stimulus. So the time of diffusion is certainly smaller than 180 milliseconds.

The calculation of the diffusion times indicated that for this case 30 - 120 milliseconds are necessary before the number of odorous molecules in the olfactory slit is below the threshold value.

Summarizing we may state that the small interruption times with which it is possible to perceive the smell sensations separately, are in good agreement with the calculated diffusion times. To get a diffusion time which is in agreement with the experimental time, it is necessary to assume for the calculations that the main part of the molecules striking the epithelium are lost the first time they touch the olfactory epithelium.

Diffusion phenomena are important for the calculation of the fraction of the odorous molecules striking the olfactory epithelium. An essential quantity is the rate of flow of the air in the olfactory slit. Suppose this rate of flow to be V cc per second. The odorous molecules will then stay $0.15/V$ seconds in the olfactory slit because the capacity of the part of the nasal chamber surrounded by the olfactory epithelium is about 0.15 cc. The molecules diffusing during this time towards the walls, are responsible for the origin of the sensation of smell. According to formula 2, the fraction striking the walls is represented by

$$1 - e^{-\frac{80 \times 0.15}{V}} = 1 - e^{-\frac{12}{V}} . \qquad (3)$$

The concentration of the odorant in the air passing the olfactory slit is not equal to the concentration of odorous molecules in the air entering the nose. The molecules striking the wall of the nasal chamber before the olfactory slit is reached are probably lost. (According to Zwaardemaker (14) most odorants are absorbed by water surfaces.) In the lower part of the cavity, the adsorption is much smaller than in the upper part because the width of the chamber and the rate of flow in the lower part is larger. The absorption of odorous molecules takes place mainly in the upper part of the cavity, therefore.

In the upper part of the chamber, the rate of flow V_1 does not differ much from the rate of flow V in the olfactory slit. The assumption that the absorption of the molecules before the olfactory slit is reached, occurs in a space with a length of 1.5 cm and a width of 1 mm seems reasonable. According to formula 2, the fraction of the molecules reaching the olfactory slit is equal to

$e^{-\frac{80 \cdot I}{V_1}}$, where V_1 represents the rate of flow in the area in which the absorption occurs and I the volume of this area. This volume is 0.15 cc. As V_1 is about V, the fraction of the molecules, reaching the olfactory slit is

$$e^{-\frac{80 \times 0.15}{V}} = e^{-\frac{12}{V}}. \qquad (4)$$

Thus the fraction of the molecules striking the epithelium of the olfactory slit is equal to

$$e^{-\frac{12}{V}} \cdot (1 - e^{-\frac{12}{V}}).$$

For small rates of flow the number of molecules striking the sense organ becomes very small because nearly all molecules are being absorbed in the mucous membrane (factor $e^{-\frac{12}{V}}$). For very large rates of flow the fraction striking the sense organ becomes small again because only a small part of the molecules in the olfactory slit can diffuse to the walls. Between both areas an optimal value exists. Although for large rates of flow the fraction of the molecules striking the walls decreases, the total number of molecules contacting the olfactory epithelium reaches a constant value (see below).

If V is the rate of flow of the air passing the olfactory slit, the odorous molecules striking the olfactory epithelium per second are contained by a volume which is given by

$$e^{-\frac{12}{V}} \cdot (1 - e^{-\frac{12}{V}}) \cdot V. \qquad (5)$$

This volume can be calculated for different rates of flow. For very large rates of flow the volume becomes constant because the odorous air reaches the olfactory slit without losses.

We shall consider the inspiration of air with rates of flow of 200, 400, 800 and 1600 cc per second. Through one nasal chamber passes 100, 200, 400 and 800 cc per second. At first we shall ignore the fact that the fraction of the air passing the olfactory slit depends on the rate of flow and assume that 7 percent is passing the slit. In formula 4 we have to substitute then for V: 7, 14, 28 and 56 cc per second. The odorous molecules which strike the olfactory epithelium of each nose side are now contained by a volume which is for a rate of flow of

200 cc per second	0.18 × 0.82 × 7	= 1.0 cc per second.
400 cc per second	0.42 × 0.58 × 14	= 3.4 cc per second.
800 cc per second	0.65 × 0.35 × 28	= 6.4 cc per second.
1600 cc per second	0.80 × 0.20 × 56	= 9.0 cc per second.

So according to our calculations the ratios of the threshold concentrations are 1 : 1.4 : 2.6 : 9.0. For the rates of flow considered, Le Magnen (18) has determined the relation between the threshold concentrations. He states for this relation 1: 2 : 8 : 16. The differences between the experimental and calculated values are probably caused by the fact that the fraction of the air passing the olfactory slit is not constantly 7 percent. For larger rates of flow this fraction becomes larger; according to formula 5 the differences between experimental and calculated threshold concentrations are becoming smaller then.

We can conclude now that for normal breathing the number of molecules reaching the olfactory epithelium of one nose side per second is contained by about 4.4 cc. This is less than 2 percent of the total number of odorous molecules inhaled.

When using the injection method, the stimulus times and injected volumes are exactly known. Unfortunately it is not possible to use large rates of flow with this method, because a sensation is produced by pure air alone then. (See 2.3.)

First we shall calculate the percentage of the injected molecules striking the olfactory epithelium for the rates of flow of 35, 70, 130 and 270 cc per second (monorhinic injection). Compared with normal breathing, the air is somewhat better directed to the top of the nasal chamber. An assumption that 10 – 15 percent of the air injected passes the olfactory slit seems reasonable. Assuming 15 percent, the molecules touching the olfactory epithelium per second are contained by a volume of:

0.10 × 0.90 × 5.2	= 0.47 cc,	when 35 cc per second is injected,
0.32 × 0.68 × 10.5	= 2.2 cc,	when 70 cc per second is injected,
0.54 × 0.46 × 19.5	= 4.7 cc,	when 130 cc per second is injected,
0.74 × 0.26 × 40.0	= 8.0 cc,	when 270 cc per second is injected.

So the ratios of the calculated threshold concentrations are 1 : 1.6 : 3.6 : 17.

To check the calculations the threshold concentrations have been determined for different rates of flow. The results are plotted in fig. 10.

Here the minimum number of injected molecules per second nec-

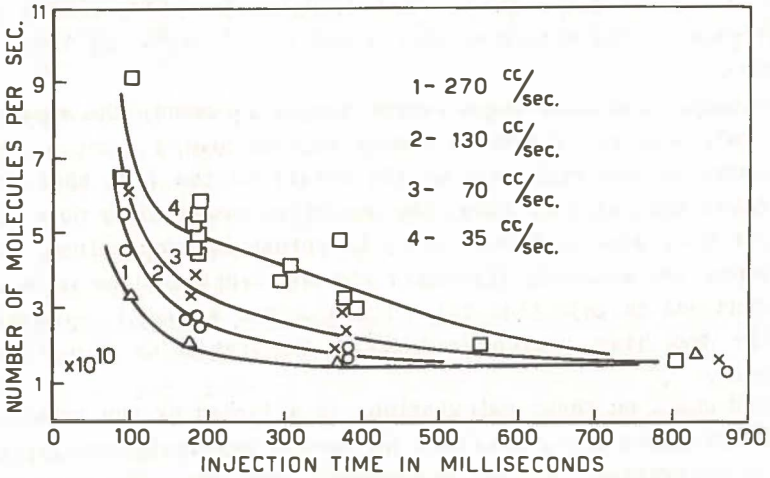


Figure 10
The relation between the injection time and the absolute threshold, expressed in number of molecules injected per second, for different rates of flow. The odorant was secondary butyl mercaptan. No corrections were applied.

essary to perceive a smell sensation is given as a function of the injection time *).

As discussed already in 2.3, there is a loss of odorous molecules because part of the injected volume is lost. The influence of this loss can clearly be seen in the course of the curve for a rate of flow of 35 cc per second. The raising of the threshold, caused by this loss is largest for small injection times because the injected volume is small then. In section 2.3 the loss was calculated and proved to be about 2 cc. So for an injection time of 200 milliseconds and a rate of flow of 35 cc per second the experimental threshold has to be divided by a factor 1.4 to find the correct threshold. For a rate of 70 cc per second this correction is nearly a factor 1.2. Taking these corrections into account the ratios of the numbers of molecules per second at threshold are about 1 : 1.3 : 1.5 : 2.1 for rates of flow of 270, 130, 70 and 35 cc per second respectively. The ratios of the threshold concentrations are 1 : 2.6 : 6.0 : 16.1 therefore **).

*) The number of molecules per second may be compared with the light intensity in the case of the eye.
**) For m-xylene the ratio of the concentrations is 1 : 2 for rates of flow of 200 cc and 100 cc per second. See 2.3.

Here the differences between the experimental and calculated ratios (1 : 1.6 : 3.6 : 17) are not significant; the fraction of the air passing the olfactory slit seems to be rather constant in this case.

When using injection times larger than 0.5 second, the experimental ratios of the threshold concentrations are: 1 : 2 : 4 : 8. This change in the ratio may be the result of the fact that for large times and rates of flow, the sensation produced by pure air alone can have some influence upon the thresholds determined. For this reason the measured threshold for an injection time of e.g. one second and an injection rate of 270 cc per second is probably a little too high, which influences the ratio to the other thresholds.

A good check on these calculations is afforded by the relation between threshold concentrations for normal breathing and injection. We determined from our experiments with the model that the percentage of the inspired air passing the olfactory slit is about 7 percent. Taking into account diffusion and absorption phenomena we calculated that the molecules contained in 4.4 cc strike the olfactory epithelium of one nose side for normal inspiration. For injection with a rate of flow of 100 cc per second this volume is 3.7 cc.

From three observations with secondary butyl mercaptan and m-xylene the threshold concentration for injection during one second with a rate of flow of 100 cc per second appeared to be on an average 1.9 as large as for normal inspiration in a current of 500 cc per second. According to our calculations one may expect a factor of 1.2. This factor is smaller than the observed value (1.9) which is probably due to the fact that the injection was monorhinic whereas inspiration dirhinic. Elsberg (18) states that the threshold for monorhinic injection is somewhat higher than for dirhinic.

Summarizing we may state that for normal inspiration approximately 2 percent of the molecules touch the olfactory epithelium. For injection with a rate of flow of 100 cc per second this part is about 4 percent.

3.4. *The number of molecules per sense cell and the number of receptors acting together at threshold stimulation*

It is a well known fact that the number of perceptible odorous molecules varies with different subjects. To get some insight in-

to these differences, the threshold concentrations for 4 different mercaptans have been determined for five subjects. The subjects were chosen at random. For the same odorant the threshold determinations have been performed as much as possible on the same day. The results of the threshold determinations are represented in table 1.

Table I

Threshold concentrations for normal breathing. The subjects are marked by I....V. In order to obtain the threshold concentrations in molecules per cc the numbers in the table must be multiplied with the corresponding numbers at the right side.

	I	II	III	IV	V	
Isobutyl mercaptan	1	6.1	9.0	20.2	47.3	$\times 4.4 10^{8}$
Allyl mercaptan	1	0.7	1.0	2.7	5.3	$\times 6.0 10^{7}$
Isopropyl mercaptan	1	4.0	5.2	7.5	5.7	$\times 2.0 10^{7}$
Secondary butyl mercaptan	1	1.3	7.6	35.7	93.4	$\times 1.3 10^{7}$

The sensitivity of the 5 subjects (I-V) for all substances investigated decreases in the order of succession of I to V. There are only a few exceptions to this rule. It should be possible that the differences are partly the result of differences in anatomical structure of the nose. However, the sensitivities vary a factor 7.5 for allyl mercaptan and a factor 93 for secondary butyl mercaptan maximally. So there are probably large differences between number or composition of the sense cells active for this group of substances. For that reason we shall assume that for normal inspiration the fraction of the air passing the olfactory slit is about the same for all subjects and that the number of molecules striking the olfactory epithelium is always 2 percent of the number of molecules inspired.

For the mercaptans the lowest threshold concentrations determined are 10^{7} molecules per cc for the subjects examined. As for normal breathing the inhaled volume is 500 cc per second, the number of molecules striking the olfactory epithelium per second is about 5.10^{7} in one nasal cavity.

As discussed in chapter II, the stimulus time is very important. For normal inspiration the stimulus time is about 1 second. When using a stimulation time of 0.15 second, or shorter, the total number of molecules required for a threshold stimulation is 6 times smaller than for a stimulation time of 1 second. So under this condition the total number of molecules required is about

9.10^6. These molecules strike the epithelium which contains about 2.10^7 sense cells.

What happens to the molecules striking the olfactory epithelium? In 3.3 we have stated that the molecules striking the epithelium generally have no opportunity to leave the surface of the epithelium. Do the molecules out of a large area concentrate on a few specific sense cells? According to Kolmer (12) the epithelium is covered with a thin water-layer. The odorous molecules have to diffuse to the sense cells through the layer of water *).

The distance between the sense cells averages about 4μ, whereas in 100 milliseconds a molecule would diffuse over a distance of about 17μ according to the formula: $x^2 = \sqrt{4\,Dt}$, where t is the diffusion time, D the coefficient of diffusion and x^2 the average square distance. For the coefficient of diffusion a value of 7.10^{-6} has been used.

As discussed on page 39, the smell sensation disappears very soon when inspiration or injection is stopped. The interval between cessation of injection or inspiration and disappearance of the smell sensation is a few tenths of a second. This is partly caused by the fact that the molecules take some time to diffuse to the walls of the olfactory slit. Some time is also needed because the molecules in the epithelium surfaces diffuse to the sense cells. It is clear from the short time in which the sensation disappears and the above calculation that the molecules cannot diffuse over a much larger distance than the distance between two sense cells. Therefore one sense cell is likely to get only the molecules striking the epithelium in the direct neighbourhood.

In our case, with 9.10^6 molecules upon 2.10^7 sense cells, every two cells get on the average one molecule. We shall prove now, that a receptor needs less than 10 molecules to be activated. It is necessary then to calculate the probability that one or more of the receptors of the whole group of 2.10^7 cells gets more than 10 molecules. If a sense cell receives on an average "half a molecule", the chance of the sense cell to receive 10 molecules is presented by $p_{10} = \frac{(1/2)^{10} \cdot e^{-1/2}}{10!} = 2.10^{-10}$. The probability

*) We have also considered the possibility of migration over the water surface (see: De Boer (19)). Many odorous substances dissolve in water, however, so surface migration may be only important for a few substances. Then the question remains how it is possible that a molecule can find the correct receptor, which is at some depth below the waterlayer.

of a receptor to receive more molecules is much smaller, e.g. 22 times smaller for p_{11} and 528 times for p_{12}. One may state therefore that the probability of a sense cell to receive at least 10 molecules is 2.10^{-10}. The chance that each of the sense cells of the whole group of 2.10^{7} receptors receives less than 10 molecules is $(1 - 2.10^{-10})^{2.10^{7}} = 1 - 4.10^{-3}$. So the probability that one or more of the sense cells of the group gets 10 or more molecules is 4.10^{-3}. An analogous calculation gives that the probability of one or more of the receptors to receive more than 8 molecules is 8.10^{-2}. This is still somewhat smaller than the actual probability of smelling for this concentration. Consequently a receptor needs 9 molecules or less to respond.

Assuming 20 receptor types, the probability that one or more of the sense cells of one receptor type receives more than 9 molecules becomes $1 + (1 - 2.10^{-10})^{10^{6}} = 2.10^{-4}$. Calculating the same probability for 7 molecules or more, a probability is obtained which is about equal to the actual probability of smelling. Consequently a receptor needs in this case 7 molecules or less to respond.

We had assumed, however, that all molecules striking the olfactory epithelium reach the sense cells and that no molecules are lost in other parts of the epithelium. This assumption is very unlikely. So the mean number of molecules striking one sense cell is very probably smaller than a half.

Neuhaus (11) indicated that for the dog only one molecule butyric acid is necessary to activate a sense cell. For the dog one molecule of mercaptan is probably also sufficient to activate one sense cell. According to our investigations and calculations, the number of mercaptan molecules necessary to activate a sense cell in man is certainly smaller than 10 and probably even 1 for the most sensitive mercaptans.

A second question is the number of sense cells to be activated before a smell sensation is perceived. We shall assume that on an average N molecules are striking the olfactory epithelium. Assuming an efficiency b, bN molecules are activating the sense cells. All the sense cells are probably not sensitive for the substance used, especially in the neighbourhood of the threshold. Hainer assumes about 20 receptor types (20). This seems a reasonable number. So about 10^{6} sense cells get $\frac{1}{20}$. bN molecules. Molecules striking the other sense cells are probably lost, because there is no time to diffuse to active sense cells.

As discussed in 2.2, there is no sudden transition for the sense of smell between perceiving an odour or not, when the num-

ber of odorous molecules striking the sense organ is decreased. For a given concentration of the odorant the percentage of positive responses may be e.g. 30 percent, for a somewhat larger concentration say 70 percent. If one determines the percentage of positive responses for various concentrations, a frequency of smelling curve is obtained. One of the possibilities is that the shape of this frequency of smelling curve lies in the variation of the sensitivity of the sense organ. It is also possible, however, that the smooth transition is caused by statistical fluctuations of the stimulus, i.e. in the number of molecules.

In vision, the assumption that the frequency of seeing curve is caused by fluctuations of the number of quanta striking the eye, has led to the conclusion that 2-4 rods have to act together simultaneously before a sensation is perceived.

We shall assume here also that the frequency of smelling curve is caused by statistical fluctuations in the number of molecules striking the sense organ, and try to determine from the steepness of the curve the number of sense cells which have to act together before a sensation is perceived.

At first we shall assume that a sense cell gives a response when only one molecule (or more) strikes the receptor and that one activated sense cell is sufficient to give a sensation. We have assumed 20 receptor types, the total number of molecules striking the sense cells being bN. The chance f that the whole group of one receptor type gets one or more molecules is represented by $f = 1 - e^{-1/20 \cdot bN}$.

If it is necessary that at least m sense cells are activated simultaneously before a sensation is perceived, and assuming that one sense cell responds already to one molecule, the chance f that a smell sensation is perceived (neglecting the very small chance that one receptor gets 2 or more molecules) is given by:

$$f = 1 - e^{-1/20 \cdot bN} \left(1 + 1/20 \cdot bN + \ldots\ldots + \frac{(1/20 \cdot bN)^{m-1}}{(m-1)!}\right) \quad (6)$$

The steepness of the curve is dependent on m. Figure 11 shows a few curves.

The part of the curve between about 20 and 70 percent of positive responses is nearly straight. A characteristic variable of the curves is the ratio s with which the number of molecules has to be increased for an increase of the frequency of smelling from 20 up to 70 percent. s is a measure for the steepness of the curve. The dependence of s on m, calculated from formula (6), is shown in fig. 12.

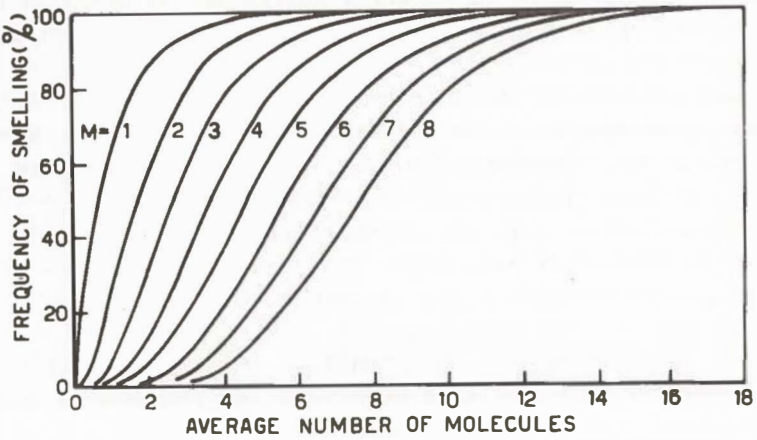


Figure 11
The theoretical relationship between the frequency of smelling and the average number of active molecules striking the entire group of sense cells, assuming that one sense cell responds to one or more molecules where *M* sense cells have to be activated simultaneously for a sensation to be perceived.

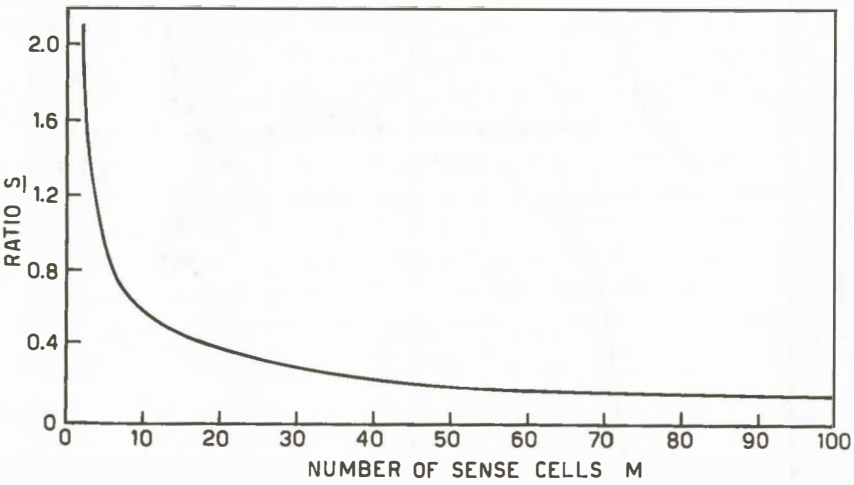


Figure 12
The theoretical relationship between the ratio *s*, with which the number of molecules has to be increased for an increase of the frequency of smelling of 20 to 70 percent, and the number of sense cells *M*, which have to respond simultaneously before a sensation is perceived.

Analogous frequency of smelling curves are obtained if more than one molecule is necessary to activate a sense cell. The curves are even steeper then.

In our experiments the dependence of the frequency of smelling on the concentration of the odorant has been determined. Some of the results are represented in fig. 13. The experiments are performed for normal inspiration. The subject inspires once per minute in a current of air, the concentration of the odorant in this current is changed at each trial. For check stimuli with no odorous substance are also given. Normally the number of responses for pure air is lower than 5 percent; runs with a higher response for zero stimulation have been omitted. When the subject cannot decide whether he perceives a sensation, the response is counted

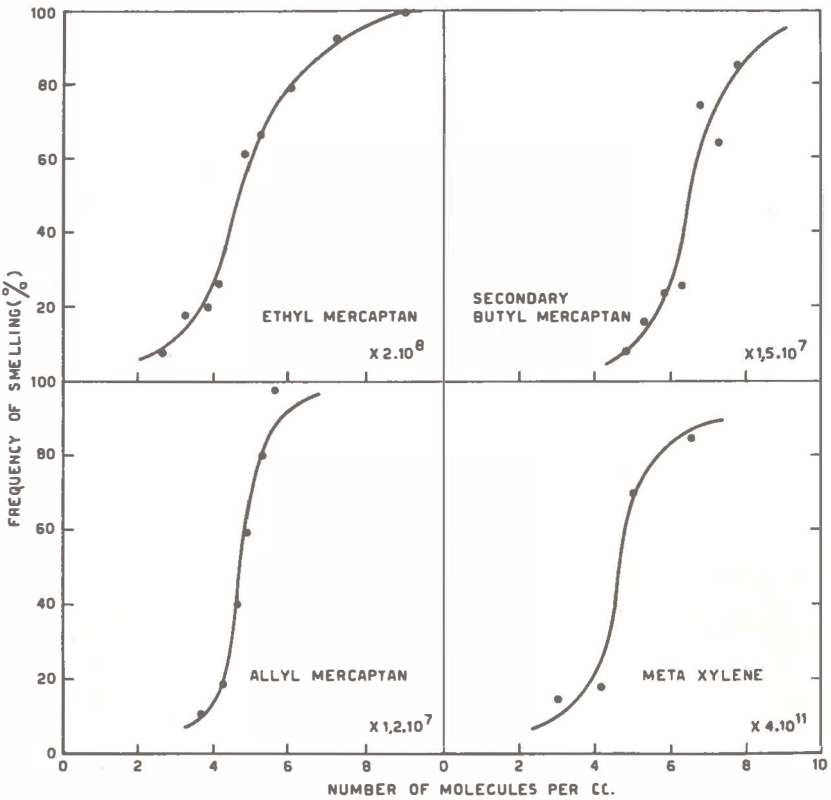


Figure 13
Experimental curves for normal breathing representing the relation between the frequency of smelling and the concentration of the odorant. For the curves about 20 trials at each concentration were made.

for half positive and for half negative. If these doubtful cases are frequent, e.g. more than 20 percent, the whole experiment is repeated. The apparatus used was described in Chapter I.

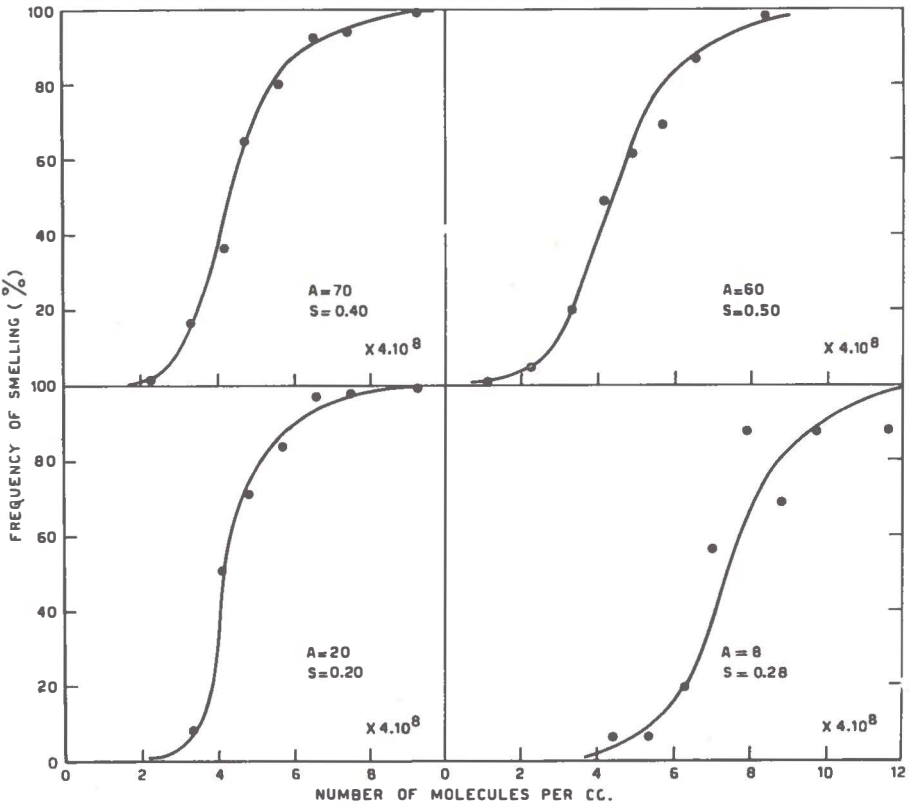
For normal inspiration the stimulation time is about one second. Using the injection method the stimulation time can be shortened. For secondary butylmercaptan a shortening of the stimulus duration to 0.10 second has no detectable influence upon the steepness of the frequency of smelling curve. Of course the number of molecules necessary for threshold stimulation is smaller for shorter times. (See 2.4). The slope of the curve greatly depends on fluctuations in the sensitivity of the subject. To prevent adaptation more than one observation per minute is not allowed. To obtain an accurate curve a large number of observations is necessary. So the experiment takes a long time, and variations in the sensitivity of the subject may occur. The curve will become flatter then. A clear example is shown in fig. 14; the substance used is ethyl mercaptan. For 70 trials per concentration the steepness s is smaller than for 20 or 8 trials per concentration.

For this reason we restricted ourselves later on to about 10 – 20 observations for one concentration of the odorant. In the following table the fraction s by which the concentration has to be increased to get an increase of the frequency of smelling from 20 to 70 percent is given. The results stated in the table were mainly obtained with the author as subject.

Table 2

odorant	threshold concentration for normal inspiration	ratio s
allyl mercaptan	6.10^{7} molecules/cc	0.15
secondary butyl mercaptan	1.10^{8} molecules/cc	0.19
isopropyl mercaptan	1.10^{8} molecules/cc	0.24
isobutyl mercaptan	4.10^{8} molecules/cc	0.21
tertiary butyl mercaptan	6.10^{8} molecules/cc	0.17
thiophenol	8.10^{8} molecules/cc	0.23
ethyl mercaptan	1.10^{9} molecules/cc	0.24
1:3 xylen-4-ol	2.10^{12} molecules/cc	0.33
m-xylene	2.10^{12} molecules/cc	0.19
acetone	6.10^{13} molecules/cc	0.26

The average value of the steepness s is 0.22. If one expects that the most sensitive odorants need a smaller number of sense



Figuur 14
Four frequency of smelling curves, showing the dependence of the steepness *s* of the curve on the number of trials *A* performed for each concentration. All the curves are determined for the same subject, the odorant used was ethyl mercaptan.

cells activated simultaneously before a sensation is perceived, according to our theory the ratio *s* should decrease when the threshold increases. The value of *s* in the table indicate that the steepness decreases by no means with increasing threshold.

For all substances used the fraction *s* does not differ significantly. If one assumes that 1 molecule is sufficient to activate one sense cell, which is probably the case for the most sensitive mercaptans, then about 40 sense cells have to be activated simultaneously to get a value for *s* of 0.22 (see fig. 12). When it takes more than one molecule to activate a sense cell, a lower number of simultaneously activated sense cells is possible.

The fluctuations in the sensitivity of the subject, and the

fluctuations in the concentration of the odorant due to experimental inaccuracies are probably the cause that the differences in the steepness s, which one expects according to the views presented above, are not observed. Perhaps the real curves are even steeper. We can only state that *at least* 40 sense cells have to be activated simultaneously, if one assumes that one molecule is sufficient to activate one sense cell.

So the number of molecules which are active in the olfactory process is at least 40. If one assumes that for the most sensitive mercaptans a hundred molecules are required to activate the sense cells, then $1/20 \cdot bN = 100$. As N is about 10^8 molecules, the efficiency b is equal to 2.10^{-5}. This is a very small efficiency. Perhaps much more sense cells than a hundred have to respond simultaneously before a sensation is perceived. There is also the possibility that a sensation is perceived only when the activated sense cells are situated in a small area, analogous to the eye, which needs about 2 – 4 quanta on 0.01 mm^2 within 0.02 second to perceive a sensation.

So the efficiency, which is defined here as the ratio of the number of molecules required to activate the sense cells and the number of molecules striking the group of sense cells, may have a reasonable value for mercaptans. Using m-xylene, the threshold concentration is about a factor 10^4 higher. The efficiency b is then very small if one assumes that only a hundred molecules are required. Probably the number of molecules necessary to activate one sense cell is much larger than one. Yet a small efficiency is not out of the question.

An analogous problem concerning statistical fluctuations arises with the determination of the perceptible difference ΔI of two smell intensities. The absolute, physical limit for this discrimination is determined by the statistical fluctuations in the number of molecules striking the olfactory epithelium. If the sensitive receptors receive $1/20 \cdot bN$ molecules on the average, the fluctuations in this number are of the order of $\sqrt{1/20 \cdot bN}$. The fluctuations in the difference are of the order of $\sqrt{1/10 \cdot bN}$. Consequently it will be impossible to detect a difference that does not exceed these fluctuations. The limit given to the Weber fraction

$$\frac{\Delta I}{I} = \frac{1}{\frac{1}{2}\sqrt{1/10 \cdot bN}} . \qquad (7)$$

Zigler and Holway (21) conducted some experiments with the olfactometer of Zwaardemaker to determine the just perceptible

differences. According to formula (7), $\log \Delta I/I = \frac{1}{2} \log I + a$ constant because $1/10 \cdot bN$ is proportional to I. In fig. 15 the results obtained by Zigler and Holway are stated in terms of $\log \Delta I/I$ and $\log I$.

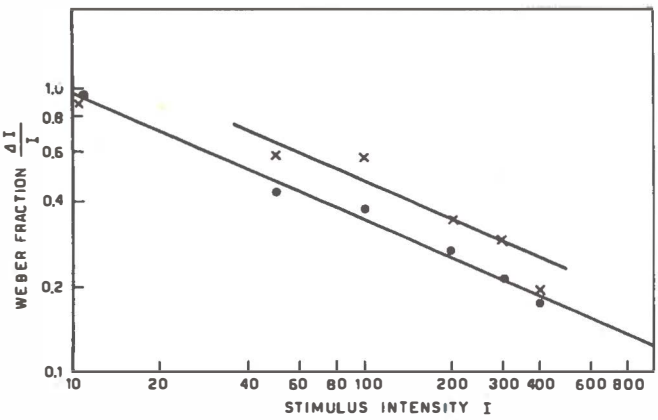


Figure 15
The relationship between the Weber fraction $\Delta I/I$ and the stimulus intensity I. Data from Zigler and Holway.

The curves are in reasonable agreement with the theory. The experimental results are for India rubber. The threshold for this odorant is not given in molecules per cc.

When using butyric acid, the Weber fraction is equal to 0.3 when about 10^{10} molecules strike the olfactory epithelium per second (Neuhaus (22)). If the curves in fig. 15 are determined by the statistical fluctuations in the number of molecules striking the olfactory epithelium, one expects that about 20 molecules are responsible for activating the receptors. So here too a very low effeciency of 5.10^{-7}. For the dog, the Weber fraction is 0.3 when about 10^5 molecules are striking the epithelium. Here the efficiency is perhaps a factor 10^5 larger.

To understand the phenomena discussed, two explanations are possible. The first explanation is that these phenomena are caused by the statistical effects of the molecules striking the epithelium. This explanation has been discussed in the present section. If it is correct, it implies that the efficiency of the receptormechanism is very small. The second explanation is that the phenomena are caused by the fluctuations in the organ itself. It is not yet possible to decide which explanation is the better one. If biological fluctuations also play a part, it is certain

that the steepness of the frequency of smelling curve can only become smaller than for statistical fluctuations alone. So the steepness due to the statistical fluctuation can only be larger than the steepness found and consequently the value found for m (40 receptors) also can only be larger than the value already calculated.

Chapter IV

THE PHENOMENON OF ADAPTATION

4. 1. *Introduction*

Adaptation is the process by which the sensitivity of the sensory system changes when the stimulus intensity is altered. The phenomenon is a very general one, occurring in all sense departments and is of great fundamental importance to the interpretation of the action of the sense organs.

When the sense organ is exposed to a continuous unvarying stimulus, the sensitivity decreases during some time and becomes constant again. When the stimulation of the sense organ ceases the organ regains its former sensitivity after some time. In vision the terms light and dark adaptation are used for these two possibilities.

For the sense of smell no terms for the two possibilities are available. As it is very awkward to describe the processes each time as adaptation to a large, or adaptation to a zero stimulus, we shall name the first process adaptation and the second recovery. Of course recovery is also adaptation.

Adaptation phenomena have been investigated extensively in psycho-physics. A well known electrophysiological phenomenon analogous to sensory adaptation is the decline in the frequency of discharge of a single afferent coming from an end organ which is subjected to prolonged stimulation. However, in many instances it is extremely difficult to translate electrophysiological results into sensory equivalents at the psychological level. This is probably the result of the fact that the sensation perceived is not based on the frequency code of a single afferent, but on a multifibre pattern of impulses which is transformed on the way go the receiving centres in the brain.

Different levels of the sensory system may display adaptation. Adaptation of the end organ, the nerves to the receiving centres in the brain and the central parts themselves is possible. A discussion of these possibilities has been given by Granit (23).

The adaptation and recovery of a sense organ can be investigated in various ways. The quantities important to the adaptation

of the sense of smell are the time during which a stimulus has to be supplied to the sense organ before the smell sensation disappears, the raising of the threshold during adaptation to a constant stimulus as a function of time and the recovery of the sensitivity of the sense organ after adaptation to a strong stimulus.

As can be seen in 4.2 the terms adaptation and fatigue are both used to characterize the adaptation of the sense organ to the supplied stimulus. Normally, fatigue indicates that processes of metabolism are incapable of acting adequately in e.g. muscular action. "Fatigue" of the sense organs is probably not based on metabolic processes. For the sense of smell it is better to use the term adaptation only; when the terms fatigue and adaptation are used both it has to be demonstrated that two different processes occur.

4.2. *Adaptation phenomena for various sense organs*

In *vision*, the first process necessary to perceive a light sensation is the absorption of light quanta in the photochemical material of the receptors. Adaptation to bright light is accompanied by a bleaching of the retina; the amount of photochemical pigment (rhodopsin) decreases. At first the origin of the changes of the sensitivity of the eye was sought in the changes of the concentration of the materials sensitive to light. The bleaching of the rhodopsin would be the only important factor. At present, however, it is generally accepted that the decrease of the sensitivity is already significant for stimulus intensities which can hardly produce any bleaching. A discussion of this problem has been given by De Vries (24, 25).

Nowadays it is clear that for low intensities adaptation takes place on another level in the visual process. The investigations of this problem led to the conclusion that the mechanism by which the first neuron is stimulated by means of products of the photochemical substance or the integrating activity at synaptic junctions is dependent on the state of adaptation.

Adaptation of the receiving centers in the brain can probably be ruled out. According to the results of Bouman (26) it is not possible to influence the sensitivity of one eye by adaptation of the other. Mandelbaum states that the dark adaptation of one eye is independent of the presence of light adaptation of the other eye. However, Elsberg and Spotniz (27) obtained different results.

Craik and Vernon (28) showed that the dark adaptation is not influenced by a temporary pressure to the eyeball applied in such a way that the eye is temporarily blind. On account of these results it may be concluded that as regards the eye, apart from the nervous mechanism in the retina and the bleaching of the rhodopsin probably no adaptation occurs.

As for the *ear* a distinction is made between "fatigue" of the ear of short duration, and the normal "fatigue", which lasts longer. The fatigue of the ear of short duration is called adaptation. It is possible that both phenomena are the results of the same reversible process. Yet the relation between adaptation and "fatigue" has to be investigated thoroughly before the differences between "fatigue" and adaptation become clear.

When using not too large intensities of short duration, the adaptation to the accoustical environment is a fast and completely reversible process.

Analogous to adaptation is the phenomenon of masking. Masking is the process by which the threshold of one tone increases when simultaneously another tone is supplied. The masking effect is most pronounced when the masking tone is strong and when the frequencies are nearly equal. Both adaptation and masking give an increase of the threshold and the processes are to such a degree analogous that, according to Lüscher and Zwislocki (29), masking and adaptation are the results of the same process.

In the case of the ear the localisation of the place where the adaptation is situated is not so difficult. From binaural experiments performed by Lüscher and Zwislocki (30) it is clear that the threshold of one ear is not raised by supplying an adaptation tone to the other ear. Harris and Rawnsly (31) indicate that adaptation does not occur in the middle ear. They come to the conclusion that the adaptation process occurs in the cochlea. It is not yet clear whether the adaptation process is of nervous origin or not.

Lüscher and Zwislocki too, come to the conclusion that for the ear adaptation is a peripheral process. They state that the adaptation process may be an adaptation of the end organ or that the auditory nerve attains equilibrium.

The origin of fatigue is not yet known.

Other receptor types also show adaptation phenomena. Matthews (32) investigated the electrophysiological properties of *proprioreceptors* of the muscles and demonstrated that the adaptation occurs mainly within 0.1 second. According to Matthews adaptation differs from fatigue. Probably the adaptation process has some

connection with the polarisation of the membrane and displacement of ions during the process, while fatigue depends on the consumption of oxygen and exhaustion of the reserves.

Adrian and Zotterman (33) a.o. investigated the adaptation of the *pressure* receptors of the skin. In this case a continuous stimulation gives a decrease in sensitivity. According to Nafe and Wagoner (34) the adaptation of the pressure receptors is caused by a decreasing of the stimulus itself. They state that pressure is felt just as long as the movement of a weight, placed on the skin surface, is maintained. When tissue resistance reduces motion to an undectable level the sensation fades out. The end point is reached, and it is obvious that complete adaptation represents stimulus failure.

The sense of *taste* is also subject to adaptation. The adaptation may proceed to such an extent that the taste sensation disappears. This phenomenon is different from the adaptation phenomena of the ear and eye. Here a sensation is always perceived, if the stimulus is large enough to give a sensation in the beginning, no matter how long the stimulus is supplied. Adaptation phenomena for the sense of taste have been investigated by Abrahams a.o. (35) and Hahn (36). The results are discussed in 4.8.

The adaptation of the sense of *smell* is well known. A person entering an environment in which a strong odorant is present will at first perceive a strong smell, but for various odorants this sensation disappears very soon. After a few minutes no smell is perceived any longer. The disappearance of the smell is called "olfactory fatigue". As many odorants have bad smells, the "olfactory fatigue" is very important. As discussed already in 4.1 it is better to use the term adaptation and not the term fatigue. In the sections below the olfactory adaptation will be discussed in detail.

4.3. *Electrophysiological investigations concerning the adaptation of the sense of smell*

To give some insight into the olfactory mechanism, the electrophysiological investigations of Adrian (37) and Ottoson (38) are very important. Adrian investigated the electrical activity of the mammalian olfactory bulb, Ottoson studied the activity of the epithelium of the frog. The latter performed some experiments concerning the capacity of the olfactory epithelium to deliver

electrical potentials after previous stimulation. When the preparation was stimulated at short intervals, the responses became successively lower, the reduction of the amplitudo related to the strength of the stimulus. Even for an interval of one minute between two stimulations, the response to the second stimulation is somewhat smaller, if large stimulations are used.

According to Adrian, the olfactory bulb of the rabbit shows potential oscillations of two kinds, viz: induced waves set up by strong olfactory stimuli and intrinsic waves due to persistent activity of the cells in the bulb. They disorganize the rapid intrinsic rhytm of the bulb and suppress the persistent discharge of impulses in favour of the olfactory discharge at each inspiration. Ultimately, however, the intrinsic activity is built up again and the persistent discharge returns, swamping the transmission of the olfactory signals. As there is no sign of failure of the receptors, under repeated stimulation at each inspiration, it is suggested by Adrian that the weakening and ultimate failure of sensation in man is due to this reappearance of the intrinsic activity after its initial disorganization.

Adaptation of the sense of smell is, according to this conception, the loss of the capacity of the olfactory signals to disorganize the intrinsic activity of the cells in the bulb. Recovery of the sense of smell is the recovery of the capacity of the olfactory signals to suppress the intrinsic activity of the bulb.

4.4. *The adaptation time required for the cessation of the smell sensation upon prolonged stimulation*

In general, the determination of the adaptation time required for the cessation of the smell sensation is hampered by various accompanying sensations. There are for instance odorous substances, which give a stimulation of taste or "heat" receptors. There are also substances which give a stinging or burning sensation. The stimulation of the trigiminus is playing a part then. Often a distinction is made between pure odorous substances and odorous substances, where the odorous sensation is accompanied by a "trigiminus sensation". Elsberg (39) investigated a series of 24 odorants by sending odorous air through the nose for some time. He found that 21 out of the 24 substances showed a stimulation of the trigiminus when large concentrations were used. So the discrimination between pure odorants and odorants with stimulation of the trigiminus does not seem convincing. Probably all

substances will stimulate the trigiminus if a sufficiently large concentration of the odorant is used. It is desirable therefore to use concentrations of the odorant with which no stimulation of the trigiminus occurs, although a well trained subject will be able to distinguish between the trigiminus and smell sensation.

In this section, we shall use the abbreviation "A.T.C.S." for "adaptation time required for the cessation of the smell sensation" because it is very inconvenient to use this long term every time.

During adaptation the smell sometimes disappears but reappears somewhat later. When once this situation is reached the smell soon disappears definitely, however. According to our experience, the sensation of the trigiminus is much longer perceptible (e.g. m-xylene).

It is obvious that the A.T.C.S. depends on the adapting intensity used. Woodrow and Karpman (2) are the only investigators who have taken into account adapting intensities in measurements of the A.T.C.S.

a) Survey of previous investigations

Aronsohn (40) conducted some experiments as early as 1886. He determined the A.T.C.S. (the adaptation time needed for the cessation of the smell sensation) of 9 odorants upon prolonged stimulation. The substances used were among others camphor, eau de Cologne and coumarin. The adaptation times required varied from 2 to 11 minutes. He also used two different concentrations of eau de Cologne. For the smallest concentration the A.T.C.S. was 1 minute; for the undiluted substance the adaptation time required was 10.5 minutes. As eau de Cologne belongs to the odorants which stimulate the trigiminus, the experiment is not conclusive.

Vaschide (41) came to the conclusion that 30 minutes adaptation to camphor is not sufficient for cessation of the "smell sensation" (for normal breathing). This large adaptation time is probably caused by the stimulation of the trigiminus, which adapts much more slowly than the olfactory organ.

Elsberg (42) investigated the A.T.C.S. at subsequent injections of odorous air into the nose. The interval between the injections was 15 seconds for one experiment, for the other 20 seconds. In the former case about 10 injections, in the latter case about 15 injections were sufficient for the disappearance of the smell. The odorant used was citral.

Woodrow and Karpman (2) are the only ones who varied the concentration of the odorant in such a way that the stimulus was

defined exactly. The A.T.C.S. (upon continuous stimulation) was determined for propyl alcohol, camphor and naphtalene. The concentration of the odorant was varied a factor 3 to 5. Different concentrations were obtained by changing the temperature of the odorant through which the air-current was sent. When the vapour pressure of the odorant at a certain temperature was twice as large as before, the number of the molecules in the stimulus was also twice as large. The threshold of the subjects was not determined however.

The results of Woodrow and Karpman indicate that the A.T.C.S. (the adaptation time required for the cessation of the smell sensation) is proportional to the adapting concentration. The relation found between this adaptation time t and the adapting intensity is $t = K + kI$, where K and k are constants and I the concentration of the odorant. It is obvious that this formula is only valid for a small range, because the A.T.C.S. is approximately zero for the threshold concentration, whereas the formula assumes the smallest possible time to be K. For the substances used K was found to be 11.5, 28 and 48 seconds, this is significantly above zero, which implies that the formula of Woodrow and Karpman is not valid for small concentrations.

b) Requirements to be fulfilled

For studying the A.T.C.S. (the adaptation time required for the cessation of the smell sensation) it is necessary to express the adapting concentration in the absolute threshold concentration. So the adapting concentration is given for instance as 100 times the threshold concentration. "Equal" adapting intensity for different subjects means that the stimulus intensities with the individual absolute thresholds as unit for the various subjects are equal. Only if this requirement is fulfilled it is to be expected that the same A.T.C.S. can be found for equal adapting intensities.

The measurements of Vaschide (41) and Aronsohn (40) do not meet this requirement. Aronsohn used undiluted odorous substances; Vaschide expressed the adapting intensity in quantity of odorous substance dissolved. For the experiments of Woodrow and Karpman the stimulus control was adequate but here, too, the absolute thresholds of the subjects were not determined.

c) Experiments

We have used d-octanol and m-xylene as odorants. As Elsberg

(39) states already, for normal breathing the stimulation of the trigiminus is often not perceptible. However, continuous injection of odorous air into the nose very soon gives a perceptible stimulation of the trigiminus. We have compared both methods for m-xylene. If an air-current of 100 cc per second with a concentration of the odorant 100 times the threshold concentration was sent continuously into the nose, already within a few minutes a strong burning sensation appeared. With normal breathing, a stimulation of the trigiminus is hardly detectable for a concentration a hundred times the threshold concentration. For larger concentrations, a burning sensation is also perceptible with normal breathing. This burning sensation lasts much longer than the "smell" sensation. During an experiment with m-xylene, the burning sensation was still perceptible for an adaptation time twice as long as the adaptation time required for the cessation of the "smell". A well-trained subject can distinguish between the two sensations. Yet for continuous injection the trigiminus effect is too strong in the case of m-xylene.

When d-octanol is used as odorous substance a stimulation of the trigiminus is not perceptible for continuous injection with the concentrations of the odorant used in our experiments.

The subject's experience is that it is easier to determine the instant when the smell sensation disappears for normal breathing than for continuous injection. At first sight normal breathing does not seem very suitable for the determination of the A.T.C.S. (adaptation time required for the cessation of the smell sensation). First because the sense organ gets the opportunity of partial recovery between two inspirations, secondly because the inspiration time and volume may vary. The partial recovery, however, is not important for the study of the relationship between A.T.C.S. and the adapting intensity because the influence upon the A.T.C.S. is comparatively the same for each concentration. The second reason, the variability of the inspiration time and volume, proves to be less serious as expected; for a well-trained subject the scattering of the results is small for normal breathing.

For these reasons we have chosen normal breathing for all adaptation experiments.

The experimental procedure was as follows:

First the threshold concentration of the subject, when not adapted, was determined. For this determination, the subject took one inspiration a minute in an air current of which the odorous

concentration could be varied. After determination of the absolute threshold, the concentration of the odorant in the current of air was raised a given factor. The subject breathed quietly in this air current until the smell sensation was no longer perceived. The A.T.C.S. was registered. Before the next experiment, the subject was given about one and a half hour to regain his former sensitivity. The same procedure as stated above was repeated then.

For the determination of the absolute threshold about 15 trials were sufficient. The subject could change the current of odorous air in such a manner that the air was sent into the cabin through an aperture with a diameter of 1.6 cm. During adaptation the subject breathed at a rate of 20 inspirations a minute in this air current, with the nose at about 0.5 cm from the aperture.

In figure 16 the relationship between the A.T.C.S. and the adaptation concentration of the odorant is represented. The adaptation concentration C is given in units of the absolute threshold concentration. So $C = 1$ is the absolute threshold concentration.

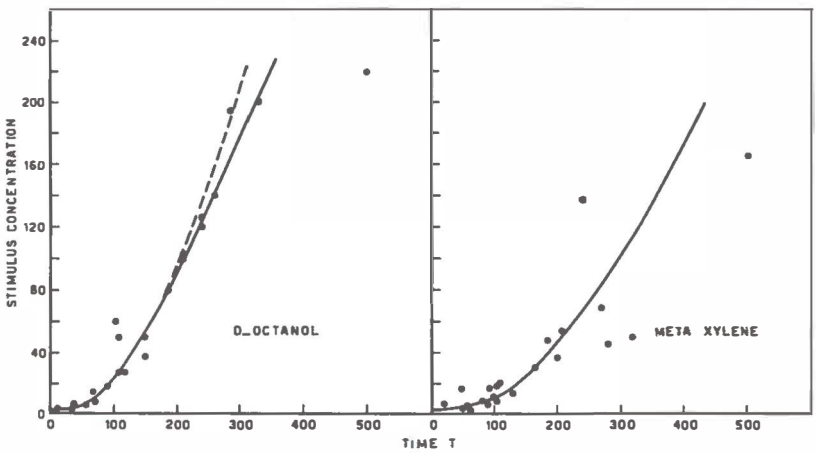


Figure 16
The relationship between the adapting concentration C and the adaptation time required for the cessation of the smell sensation for d-octanol and m-xylene. The dotted curve for d-octanol is given by $t = 20\sqrt{C - 1}$, the curve for m-xylene by $t = 30\sqrt{C - 1}$.

It is clear that for the investigated range of concentrations, the relation between the A.T.C.S. and the used concentration is not a linear one. For a stimulus intensity of 10 times the absolute threshold an adaptation time of about 60 seconds is required

when d-octanol is used. For 100 times the threshold concentration this time is 210 seconds. A fair approximation for the adaptation time t is found, if one plots t as a function of $\sqrt{C-1}$. The dotted curve given in figure 16 satisfies the equation

$$t = 20\sqrt{C-1}$$

for d-octanol. The results of analogous experiments for m-xylene are also stated in figure 16. Here the curve corresponds with the equation $t = 30\sqrt{C-1}$.

Comparing m-xylene to d-octanol, the A.T.C.S. is with equal adapting intensities for d-octanol a factor 1.5 smaller than for m-xylene. The adaptation times determined for m-xylene show a larger scattering than the times for d-octanol. This is probably the result of the stimulation of the trigiminus for m-xylene, with which it is more difficult to determine the instant of cessation of the smell.

It is impossible to compare our results directly with those of Woodrow and Karpman, because the latter made use of continuous injection. According to these experimenters, the A.T.C.S. ranges from 60 to 200 seconds for on an average a fourfold increase of the concentrations of the used odorants. According to a few experiments made by us, the A.T.C.S. required for normal breathing is about 1.7 times as large as for continuous injection. So the variations in injection time of 60 up to 200 seconds for the experiments of Woodrow and Karpman correspond for normal breathing with a variation in adaptation times of about 100 to 340 seconds. In our experiments the corresponding variations in the concentrations of the odorant are a factor 8 and 6 for d-octanol and m-xylene respectively. The differences with the results of Woodrow and Karpman are probably caused by the use of different odorous substances. In any case their results are not contradictory to ours, because our curve for d-octanol is approximately linear when adaptation times larger than 100 seconds are required, in accordance with the linearity found by Woodrow and Karpman in this range of times.

4.5. *The raising of the threshold during adaptation*

The best way to represent the change in sensitivity of the sense organ to which a prolonged stimulus is supplied is the determination of the just perceptible concentration of the odorant as a function of the adaptation time. A special case is the de-

termination of the A.C.T.S., which was discussed in section 4.4, because at the moment when the smell disappears the just perceptible concentration is equal to the concentration of the adapting stimulus supplied.

a) Survey of previous investigations

The rate of adaptation appears to be dependent on the adapting intensity. With the aid of an olfactometer some experiments have been made by Zwaardemaker (43) to investigate the influence of the adapting intensity upon the rate of adaptation. The sense organ was adapted to a certain stimulus intensity, which was stated in terms of exposure of the odorous substance in cm. The raising of the threshold was determined with the aid of the same olfactometer by changing the surface of the odorous substances exposed. As discussed already in chapter I, the amount of odorous substance exposed is perhaps not completely proportional to the stimulus intensity. Small deviations are possible, therefore. The results of the experiments of Zwaardemaker are given in figure 17.

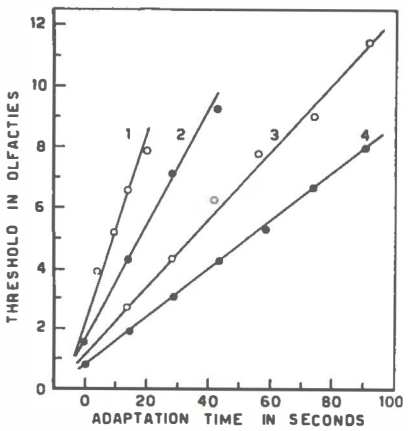


Figure 17
Olfactory adaptation to two concentrations of benzoin and India rubber; according to Zwaardemaker. The adapting intensities for benzoin were 9 and 3.5 olfacties respectively (curves 1 and 2); for India rubber 14 and 10 olfacties (curves 3 and 4).

In figure 17 the raising of the threshold as a function of the adaptation time is given for two different stimulus concentrations of benzoin and India rubber. In the case of benzoin, to which adaptation is more rapid, a concentration 9 times the

threshold value produces a fourfold heightening of the absolute threshold in 5 seconds. The time required for an equal raising of the threshold, when a stimulus intensity of 3.5 times the absolute threshold is used, is 3 times longer. Analogous results can be deduced from the curves for India rubber.

According to the curves, the raising of the threshold is linear to time. Apparently the adaptation processes have not yet been brought to completion. It is a question whether the thresholds become infinitely high or reach a constant value.

Vaschide (41) performed analogous experiments. The substances used were camphor, ammonia and ether. The results are in accordance with those of Zwaardemaker. However, Vaschide expressed the adapting intensity in quantity of odorous substance dissolved, to which the adapting intensity need not be proportional.

Komuro (44) determined the raising of the threshold for three odorants: terpineol, guajacol and capric acid. He used different concentrations of the odorants. The subject took 5 inspirations of air while the concentration of the odorant was known. The adaptation time was about 15 seconds, therefore. By means of Zwaardemaker's olfactometer the raising of the threshold was determined. The results are represented in figure 18.

Backman (45) evaporated a known quantity of odorous substance in a room. During a quarter of an hour the subject adapted completely to the odorous air in the room. After adaptation, the smallest perceptible concentration of the odorant was determined with the aid of a "camera odorata". The perceptible concentration appeared to be smaller than the adapting concentration. Komuro obtained the same result.

Zwaardemaker noticed that the difference is probably caused by a recovery of the sense organ between the two measurements. In 4.6 we shall show that this is indeed very probable because at first recovery is very fast.

Cheesman and Mayne (46) performed experiments analogous to those of Komuro. However, they too express the stimulus intensity in terms of amount of odorous substance dissolved.

b) Experiments

The aim of our experiments is to investigate the raising of the threshold when the subject is breathing in a current of odorous air in which the concentration of the odorant is constant.

The experiments started with the determination of the absolute threshold. After determination of the threshold, the subject was adapted to a given stimulus concentration, say 100 times the ab-

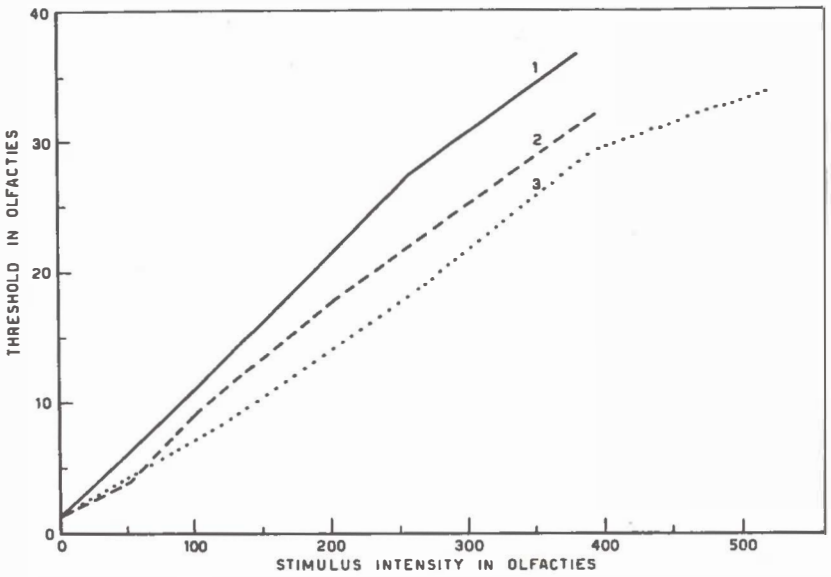


Figure 18
The raising of the threshold when adapted during 5 inspirations. The raising of the threshold as a function of the adapting intensity is given for guaiacol (1), caproic acid (2) and terpineol (3). According to Komuro.

solute threshold concentration. The problem now is the determination of the threshold concentration as a function of the adaptation time. A possibility is to cease adaptation on a given instant and to determine the threshold reached. Even for a rough determination of a threshold, however, a time of at least one minute is required. During this time, the sense organ recovers partially, and the wrong threshold is measured. Therefore we have used the following procedure:

During adaptation to the stimulus concentration, the concentration of the odorant was lowered suddenly. For this test concentration the subject had to state whether he smelled this concentration or not. After this measurement, the original adaptation intensity was supplied again to the subject. If the subject smelled the test concentration of the odorant, the whole trial was repeated again after some time. When for the second trial no smell was perceived for the test concentration, we took the average of the adaptation times, registered at both trials, as the adaptation time required to get a raising of the threshold concentration equal to the test concentration of the odorant. During

adaptation, various adaptation times for different odour concentrations can be determined.

Of course the odour concentrations are successively larger. We shall now describe the procedure more in detail for an adapting intensity of 100 times the absolute threshold concentration *. The used odorant was d-octanol. After 10 seconds of adaptation, the concentration of the odorant was suddenly reduced to 20 times the absolute threshold concentration. When the test concentration had been adjusted, the subject received a signal. For the next inspiration, the subject had to state whether he smelled something or not. Simultaneously, the subject read the time on the stopwatch. After the determination, the "operator" was signalled that the original concentration of the odorant (100 times the threshold concentration) could be supplied again.

It was possible to perform the entire determination, including the change of the concentration of the odorant within 8 seconds. At this trial the subject still perceived a smell sensation for a concentration 20 times the original threshold concentration. The whole process was repeated then some time later on. The moment of the next determination was fixed by the subject, because he could tell whether the smell at the former determination was strong, so that the next determination should occur somewhat later, or whether the smell was weak, so that the next determination could take place soon.

For the next determination, 20 seconds after onset of adaptation, no smell sensation was perceived by the subject. For the adaptation time required for a raising of the threshold concentration of 20 times the absolute threshold the average value of the two adaptation times was taken.

The same procedure was performed again for a larger concentration, in our case 40 times the absolute threshold concentration, and so on. The determination of the adaptation time required for a raising of the threshold to 100 times the absolute concentration was not difficult, the adaptation time for this raising was equal to the adaptation time required for the cessation of the smell for the adapting stimulus.

* All the adaptation measurements, and the main part of the other experiments described in this thesis, were performed with the author as subject.

If the same concentration (100 times the original threshold) continued to be supplied, the threshold rose above this concentration. The determination of these thresholds occurred in the same way. The results of the experiments are given in figure 19.

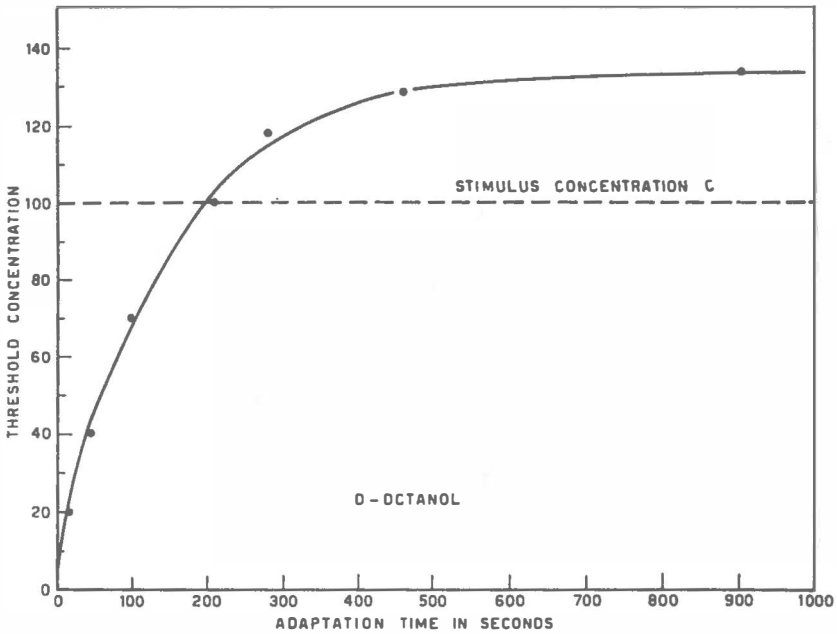


Figure 19
The raising of the threshold for normal breathing as a function of the adaptation time when a fixed adapting concentration of 100 times the absolute threshold concentration is supplied to the sense organ.

Due to the method employed, the determination of the thresholds was slightly inaccurate. First because the adaptation time for a given threshold was expressed by the average of two determinations, as discussed in our example of a raising of 20 times the absolute threshold. This error will be of the order of 5 seconds for the main part of the measurements.

A second difficulty is the fact that during the experiment the adaptation did not always occur with the correct concentration of the odorant. In our example, a raising of the threshold 100 times the original threshold occurred for an adaptation time of 210 seconds. In this time 3 other thresholds were determined. For this determination normally about 6 observations were necessary. As one observation lasted about 8 seconds, the subject was adapt-

ed for 50 seconds to a wrong concentration. However, the error in the time of 210 seconds will be lower than 50 seconds, because during change in concentration the subject adapted to a concentration between 100 times the absolute threshold and concentration of the test stimulus. It is difficult to make an estimate of the true error. It seems reasonable to state that the adaptation times are not more than 10 percent too long.

There was also the possibility that the sensitivity of the subject changed during the experiments. As the scattering of the points determined was small, the influence of sensitivity changes was probably negligible.

The raising of the threshold was determined for various concentrations, to which the subject adapted. Between two series of determinations, the subject was given at least one hour of rest to become as sensitive as before. Normally after 1 hour the threshold was still about 10 - 50 percent higher than at the first experiment. The results of the experiments are given in fig. 20.

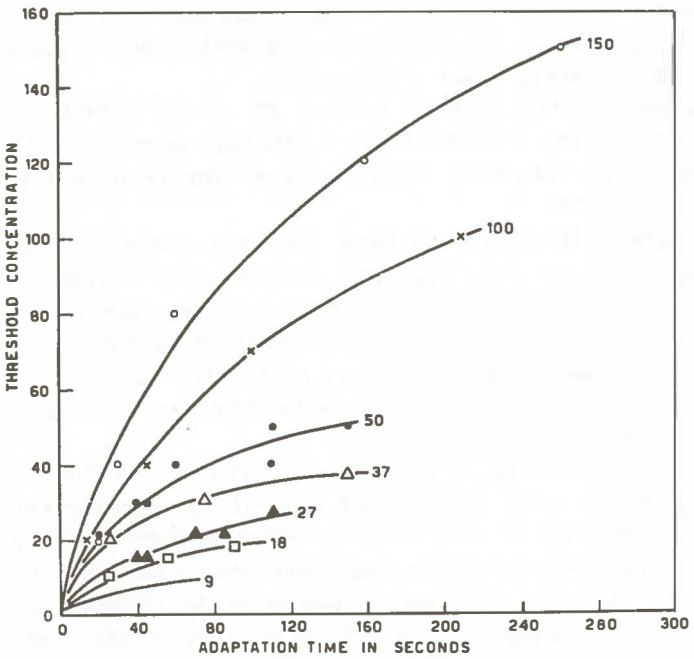


Figure 20
The raising of the threshold for d-octanol during adaptation to various stimulus concentrations. The numbers at the curves give the adapting concentrations used.

For the adapting intensities of 50 and 27 times the absolute threshold concentration 2 series of thresholds were determined. With the exception of a few points, the scattering of the thresholds was small.

The adaption was stopped when no smell was perceived any longer for the stimulus supplied. The adaptation was not continued, because otherwise the time necessary for the sense organ to recover would be even larger than 1 or 2 hours.

The raising of the threshold for m-xylene has also been determined. The shape of the curves is analogous to those of the curves found for d-octanol.

The results of Zwaardemaker, who found a linear relation between the raised threshold and adaptation time, do not agree with our results. Yet our results are more probable because it is very unlikely that a sudden transition between the region of raising thresholds and the region of constant thresholds should occur (see fig. 19).

When the sense organ adapts itself to a concentration 10 times the threshold concentration, the threshold is raised after 30 seconds a factor 6 for d-octanol and about a factor 5 for m-xylene. Analogous results of Zwaardemaker are a factor 7 for benzoin and a factor 3 for India rubber.

From the results plotted in fig. 20, it is possible to give the raising of the threshold for a constant adaptation time as a function of the adapting concentration. The results are represented in fig. 21.

The curves in figure 21 have the same shape as the curves obtained by Komuro (see fig. 18). Komuro adapted himself during about 5 inspirations so the adaptation time was about 15 seconds. The raising of the threshold, as determined by Komuro, is about a factor 2 slower than the raising of the threshold in figure 21 for an adaptation time of 20 seconds. The agreement is reasonable, therefore.

It is also possible to state the raising of the threshold as a function of the total quantity of odorous substance entering the nose, starting again from the experimental results plotted in fig. 20. The quantity of odorous substance, striking the epithelium, is a constant fraction of the quantity entering the nose. In figure 22, a quantity of 1 unit means that during 1 second the absolute threshold concentration is inspired. A quantity of 1000 units can be obtained with various adaptation times and adapting intensities, for instance for an adaptation time of 10 seconds and an adapting intensity of 100 times the absolute threshold

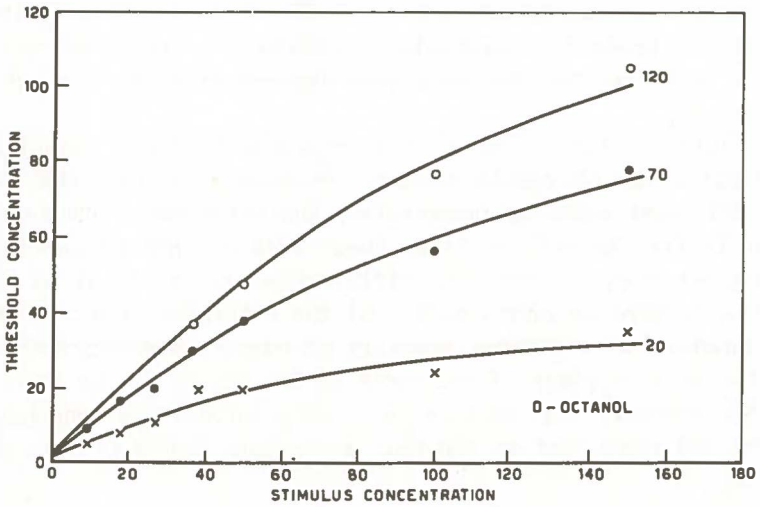


Figure 21
The raising of the threshold concentration when adapted during a fixed time to various stimulus concentrations. For each curve the adaptation time is given in the figure. The data in this figure are deduced from the curves in fig. 20.

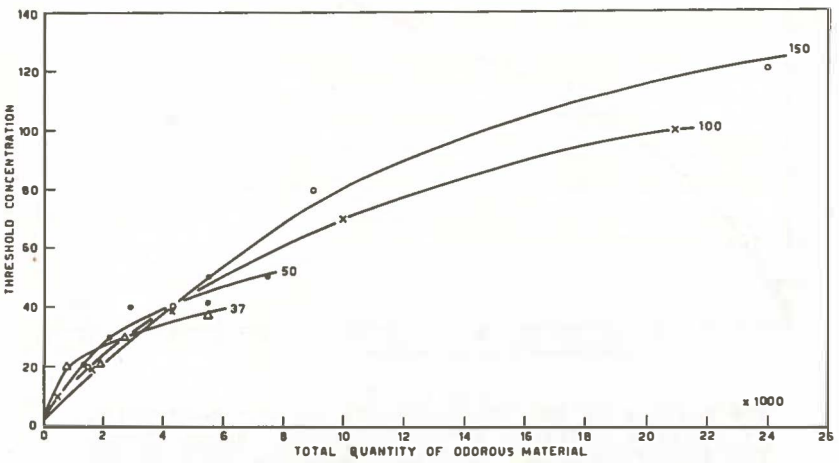


Figure 22
The raising of the threshold as a function of the quantity of odorous material supplied to the sense organ. A quantity of 1 unit corresponds with an adapting concentration equal to the absolute threshold concentration supplied for one second to the sense organ. The numbers at the right side of the curves give the concentrations used for the adaptation. The odorant used was d-octanol.

concentration or an adaptation time of 200 seconds with an adapting intensity 5 times the threshold concentration. The results are plotted in figure 22. The data were derived from the data given in figure 20.

For long adaptation times, and consequently large quantities of odorant, the threshold becomes constant (see fig. 19). When using different adapting intensities, the differences between the curves in fig. 22 will be large then. When a constant threshold has not yet been reached, the differences are small. It is then possible to give an approximation of the raising of the threshold as a function of the total quantity of odorous substance coming into the nose by means of the curve in fig. 23. It has to be borne in mind, however, that this curve is only valid if the adaptation has not yet proceeded so far that a constant threshold has been reached.

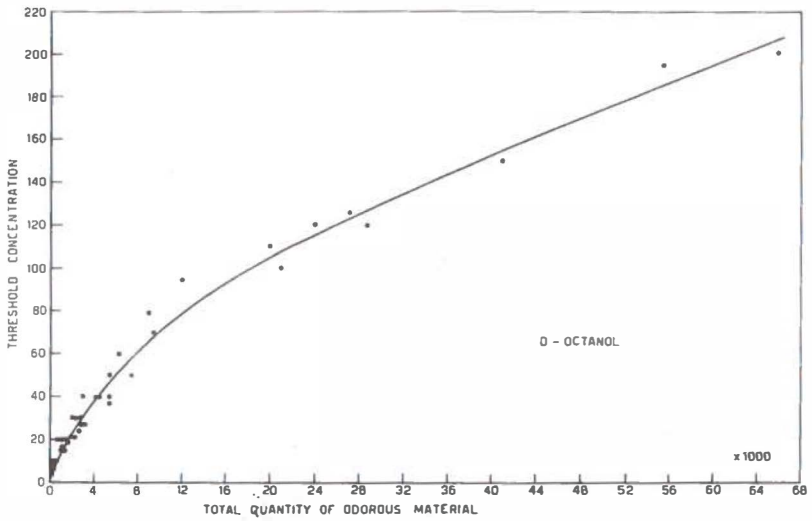


Figure 23
The raising of the threshold for various quantities of odorous material supplied to the sense organ. The differences for different adapting intensities are neglected in this figure. Adaptation has not proceeded so far that a constant threshold has been reached.

For plotting the curve the data of figure 22 are used and at the same time the data obtained from the determinations of the adaptation time required for the cessation of the smell sensation (see 4.4).

With m-xylene as odorant analogous results are obtained.

If adaptation had not yet proceeded so far that a constant threshold is reached, the raising of the threshold is approximately the same (see fig. 22) for the same total quantity of odorous material. This implies that in order to obtain a fixed raising of the threshold, the relation between the adaptation time and the adapting concentration of the odorant is expressed by a hyperbole. In figure 24 the hyperboles which agree with the total amount of odorous substance necessary to raise the threshold 15, 30 and 45 times are given.

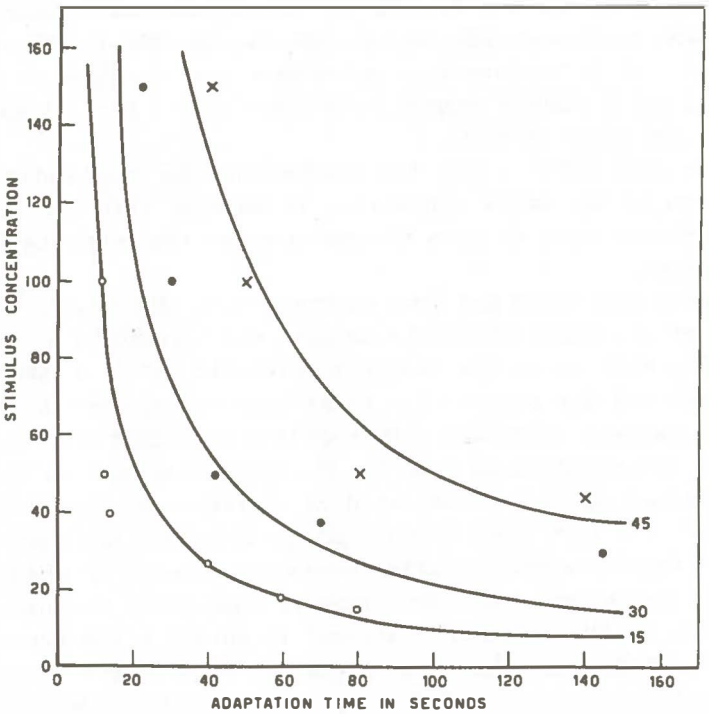


Figure 24
The relationship between adapting concentration and adaptation time, for the same raising of the threshold. The curves given are hyperboles. The numbers at the right side of the curves give the raised threshold concentration. The data in this figure are deduced from figure 20.

4.6. *The recovery of the sense of smell after previous adaptation*

a) Survey of previous investigations

As early as 1886 Aronsohn (40) performed some experiments to

investigate the recovery of the sense of smell after previous adaptation. The experiments were made with eau de Cologne and coumarin as odorants. The subject smelled at the odorants until no smell was perceived any longer. The adaptation times required were 65 seconds for eau de Cologne and 120 seconds for coumarin, both times were determined for the same subject. After a time of recovery of 1 and 3 minutes respectively the experiment was repeated. The adaptation time, required for the cessation of the smell was now 45 seconds for eau de Cologne and further 35, 25, 20, 15, 15, 25, 22, 22, 20, 22, 15, 15 seconds. For coumarin the adaptation times were 120, 65, 45, 25, 35, 20, 20, 15, 17, 10, 8, 8 seconds. It is obvious from above data that the time of recovery of 1 and 3 minutes respectively gives only a partial restoration of the sense of smell.

It is also obvious that the adaptation time required for the cessation of the smell sensation, is shorter than the time of recovery necessary to give the sense organ its complete former sensitivity.

Elsberg (42) performed some experiments to investigate the recovery of the sense organ. The subject was adapted to a stimulus intensity well above the absolute threshold until no sensation was perceived any longer. The adaptation was stopped then. The time of recovery necessary before another injection with the same odorous concentration as used for the adaptation gave an olfactory sensation again was indicated as "duration of the olfactory fatigue". The term used is misleading, because a new perception of an olfactory sensation after cessation (caused by adaptation) does not imply that the sense organ is completely restored. This "duration of the olfactory fatigue" is merely a measure of the rapidity with which the sense organ recovers in the beginning.

From the experiments of Elsberg it follows that the "duration of the fatigue" increases for larger adaptation times. Examples are the experiments for coffee. The experiments indicated that the "duration of the fatigue" was 1.5, 3.5, 4 and 5.5 minutes for adaptation during ½, 1, 2 and 3 minutes respectively, with the same stimulus intensity. The above data were for bilateral injection. With unilateral injection, the "duration of the olfactory fatigue" was shorter: 1.5, 2, 3 and 4.5 minutes respectively. Elsberg also investigated the influence of adaptation of one side of the nose upon the sensitivity of the other side. If one side was adapted until no odour was perceived any longer, the other side did not perceive a smell either when the same odour concen-

tration was used. The "duration of the olfactory fatigue" was in this case 1.5, 2, 2.5 and 3 minutes, when coffee was used as odorant and when the same injection times were used as discussed in our case. Elsberg also determined the influence of tumours of the brain upon the sensitivity and the adaptation times required for the cessation of the olfactory sensation. He came to the conclusion that adaptation is a temporary disobedience of a function of the brain.

b) Experiments

The purpose of our experiments was to determine the capacity of the olfactory system to recover from the influences of adaptation to a more or less intensive stimulation. The best method to characterize the recovery is to state the threshold as a function of the recovery time. The experiments were made as follows:

First the threshold concentration of the subject was determined when the subject was not yet adapted. After determination of the absolute threshold concentration, the subject was adapted to a given stimulus intensity. When the subject perceived no odour any longer, the adaptation was ceased. After cessation of adaptation, the subject breathed normally the pure air in the cabin, while the concentration of the odorant in the current of air was adjusted to a lower value. The subject took at intervals one inspiration in the current of odorous air and registered the recovery time necessary to perceive a smell sensation for the test concentration. The same procedure can be repeated for various, successively lower, concentrations of the test stimulus.

So with the procedure described above, the recovery time necessary before a given concentration is smelled can be determined. The adaptation occurred by inspiration in a current of odorous air at a rate of 20 inspirations per minute. The current of air was sent into the cabin through an opening with a diameter of 1.6 cm and with a rate of flow of 300 cc per second. During adaptation, odorous air came into the cabin. Consequently a good ventilation of the cabin was required. When adaptation ceased, the odorous air was normally sent outside by means of a ventilating system (see chapter I) and only for a few seconds entered the cabin when the subject took an inspiration in the air current to state whether he smelled the test concentration or not.

At first the subject took the inspirations in the current of odorous air at short intervals, because the recovery was fast then and an olfactory sensation for the test stimulus was soon perceived.

When a smell sensation was not perceived for the first but for the second trial, we took for the recovery time, required to perceive a smell sensation for a given test concentration, the average of the recovery times at two successive trials.

The results of the experiments are plotted in fig. 25.

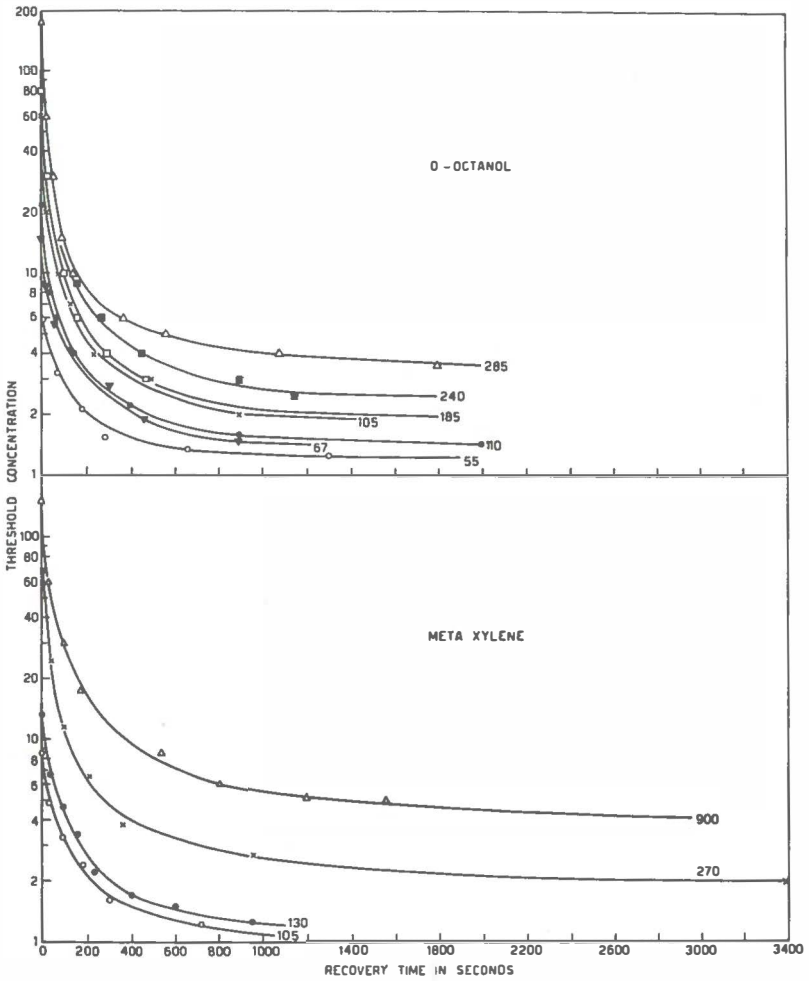


Figure 25
The relationship between the decrease of the threshold and the recovery time for various adaptation times and adapting intensities The adapting intensities are equal to the threshold concentrations when recovery begins. At the right side of the curve the adaptation times used.

At the beginning the recovery of the sensory system is very rapid, but after a few minutes much slower. The shape is the same for all curves. After about 100 seconds of recovery, the ratios of the thresholds become constant for two curves.

Some experiments were made to study this ratio in more detail. The subject was adapted to a *fixed* stimulus intensity during various adaptation times. For the recovery of the sense organ the curves in figure 26 were found.

The ratio of the thresholds after about 100 seconds of recovery is equal to $T^{1/6}$ or $T^{1/5}$, where T is the ratio of the adaptation times. We have no intention to emphasize this relation. The only essential point is that the increase of the threshold after more than 100 seconds of recovery increases only slowly with the previous adaptation time. This $T^{1/5}$ law implies that a 32-fold increase of the adaptation time produces after a few minutes of recovery only a twofold increase of the threshold.

It is also possible to use different adapting intensities and a *fixed* adaptation time. Here the ratio of the thresholds after about 100 seconds of recovery is about $I^{1/4}$, where I is the ratio of the adaptation concentrations. Using different adaptation times as well as different adapting intensities, one may expect that the ratio of the thresholds for different curves is equal to $I^{1/4}. T^{1/5}$. A factor $(IT)^{1/4}$ is a reasonable approximation. To our opinion, the only essential points of this relation are:

1. After a few minutes of recovery, the thresholds are controlled by the total quantity of odorous substance, used for the adaptation.
2. A 16 fold increase of the quantity of odorous substance only gives a twofold increase of the threshold, if the recovery time of the sensory system is more than a few minutes.

To check the first point, some experiments for a fixed quantity of odorous substance were made. Using adapting concentrations of 24, 60 and 120 times the absolute threshold concentration and adaptation times of 100, 60 and 20 seconds respectively, the thresholds, after a few minutes, did not scatter more than 10 percent around the average value. A large adapting intensity seems to raise the thresholds slightly more than a small one, if the total quantity of odorous material used for the adaptation is the same, this does not seem unreasonable.

One may conclude, therefore, that when adaptation is not yet complete, the threshold of the sense organ after a few minutes of recovery is mainly determined by the quantity of odorous substance used for the adaptation. The threshold increases much less

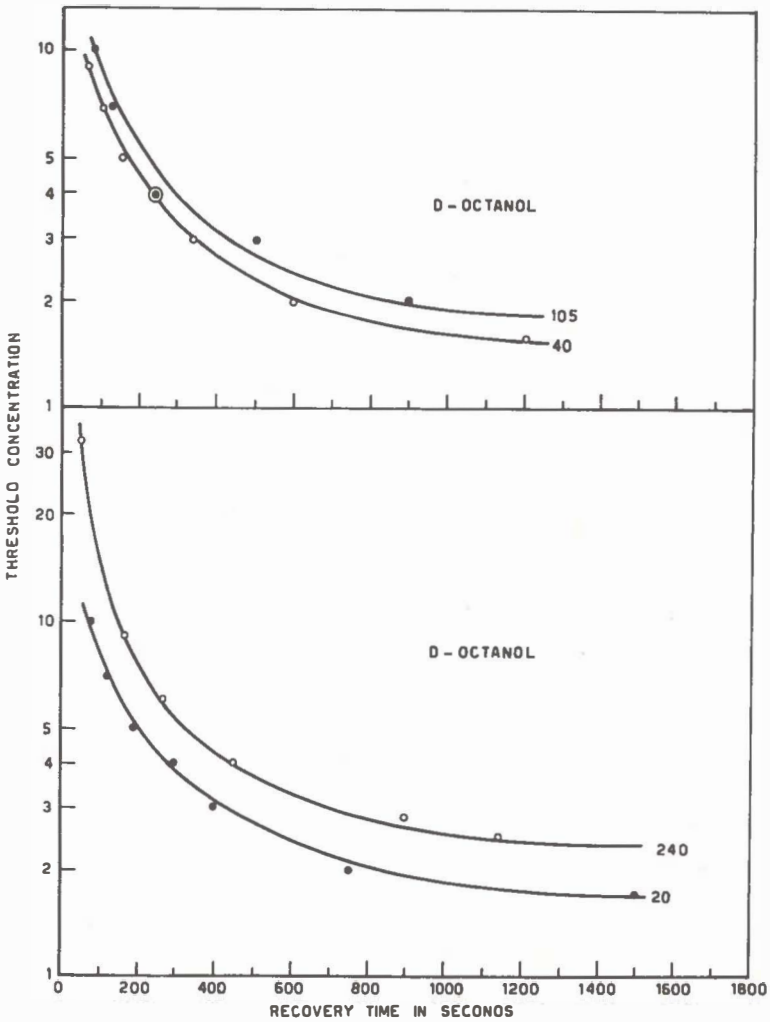


Figure 26
The recovery of the sense of smell, when different adaptation times but the same adapting intensity is used. At the right side of the curves the adaptation times. Adapting intensity for the upper curves 60 times the absolute threshold concentration, for the lower curves 120 times.

than proportionally to this total amount of odorous substance.

From the data represented in figure 25, it is possible to plot the threshold after a fixed time of recovery as a function of the total quantity of odorous material used for the adaptation. The results are represented in figure 27.

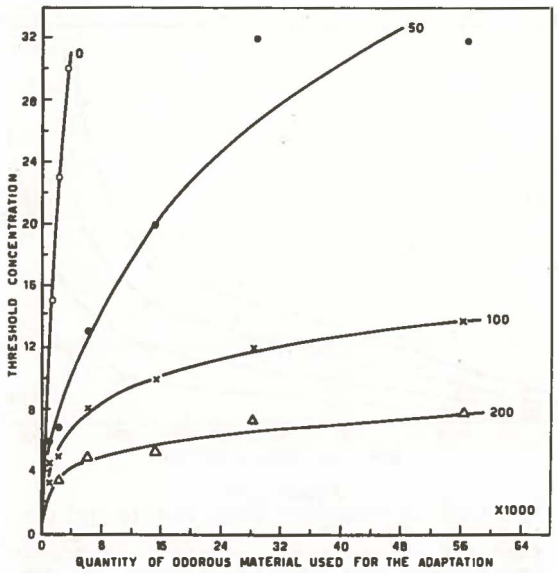


Figure 27
The threshold concentration as a function of the quantity of odorous material used for the adaptation, for the same recovery time. The recovery time is given at the curves. Odorant used was d-octanol.

It is also possible to plot the time of recovery necessary to obtain a fixed threshold as a function of the quantity of odorous substance used for the adaptation. The curves plotted in figure 28 are derived from the data given in figure 25.

The time of recovery, necessary to obtain a threshold value one half of the threshold when adaptation is stopped, is also of interest. For the various quantities of odorous material used for adaptation and with the special requirement that the adaptation was to cease when no smell sensation was perceived anylonger this time appears to be 40 seconds, except for the lowest quantity used.

For a tenfold lowering of the threshold the recovery times are not constant, as can be seen in figure 29.

To find the centres where adaptation occurs, the following experiment was made. One side of the nose was adapted during 80 seconds to a stimulus concentration of 70 times the absolute threshold concentration. The odorant used was m-xylene, adaptation occurred by monorhinic injection of an air-current of 100 cc per second. To prevent a current of odorous air passing from one

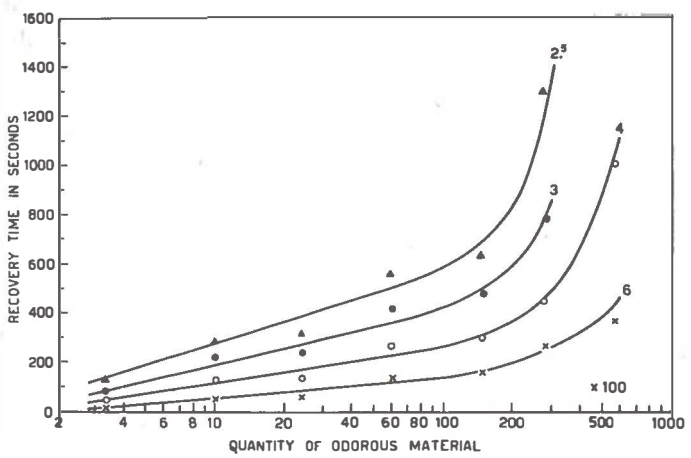


Figure 28
The time of recovery required to obtain a given threshold concentration as a function of the total quantity of d-octanol used for the adaptation. At the curves the threshold concentrations.

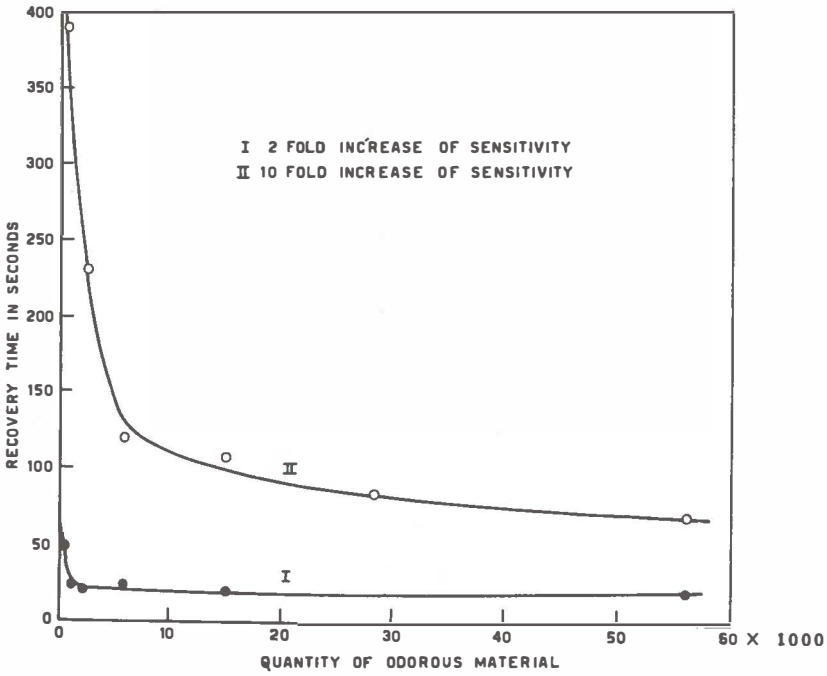


Figure 29
The recovery time required for a two- and tenfold increase of the sensitivity respectively, for various quantities of odorous material used for the adaptation. Odorant used is d-octanol.

nasal chamber into the other, the other nostril was locked by means of a wad of cotton wool. It remains possible that some odorous molecules diffuse by way of the throat into the other nasal cavity. To exclude this possibility, a similar experiment for d-octanol was carried out in such a way that during adaptation of one nose side, a current of pure air was sent through the other side. Odorous molecules certainly did not enter the nasal cavity of the nonadapted side of the nose in this case. In figure 30 the results of the experiments are given. The upper curves express the recovery of the nose to which the stimulus had been supplied, the lower curve the recovery of the other side of the nose. For larger times there is a plain difference between the curves. The unadapted side soon regains its former sensitivity, for the other side the former sensitivity is reached much later.

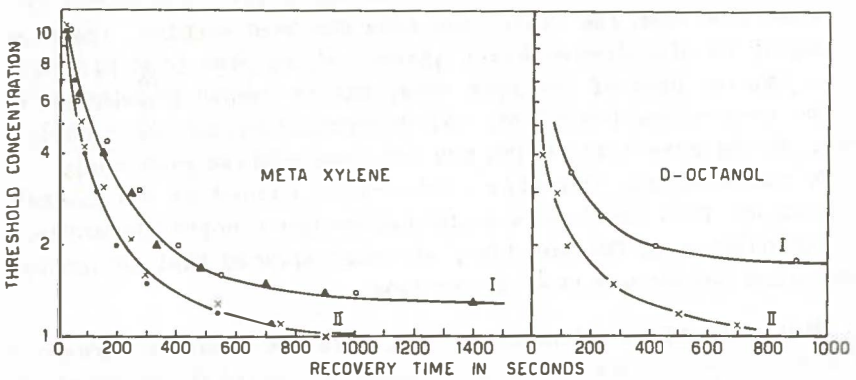


Figure 30
The recovery of one nose side when the same nose side has been adapted (I) and the recovery of the nose side which has not been adapted (II). For the m-xylene an adapting concentration of 70 times the absolute concentration was injected during 80 seconds, for d-octanol a concentration 50 times the absolute threshold concentration during 100 seconds before recovery started.

4.7. *Discussion*

I. In order to explain the adaptation phenomena, it is important to localise the level where the adaptation occurs. Possibilities are adaptation processes in the end organ or in the central parts.

Formerly it was assumed that the adaptation occurred in the end organ, in which case the adaptation would be caused by a

rapid saturation of the membranes, in general the environment, of the receptors. An argument against saturation of the receptors is that an olfactory sensation is always perceived if the stimulus concentration is raised slightly above the adapting concentration, no matter how long the adaptation lasts. (See figure 19). A receptor, which is saturated with molecules, is unlikely to respond again when the stimulus intensity is raised slightly. Neither is it probable that there is a large number of receptors with different sensitivities for the same substance.

A conclusive proof is the experiment discussed in 4.6 (figure 30), which is an improvement on the experiments made by Elsberg (see 4.6a). The experiments make it clear that the adaptation is mainly situated in the central parts of the olfactory system.

Normally, the recovery curves are composed of a fast and a slow component. However, for the recovery curves determined for one nose side when the other nose side has been adapted, the slow component is almost completely absent. It is even possible that the remaining part of the slow component is caused by adaptation to the test stimuli used for the determination of the recovery curve of the nose side, which had not been adapted previously.

To our opinion, the slow recovery is caused by peripheral adaptation. This opinion is supported by the electro-physiological experiments of Ottoson (38), who demonstrated that peripheral adaptation indeed occurred at the frog.

II. When a stimulus intensity of 100 times the absolute threshold is used for the adaptation, the raised threshold D can be given for d-octanol by formula

$$D = 1 + 134\ (1 - e^{-t/150})$$

where t is the adaptation time (see figure 19).

When the adaptation lasts much longer than 150 seconds, the threshold reaches a constant value, the ultimate value reached is about 1.35 times the concentration of the stimulus used for the adaptation.

Using small concentrations for the adapting stimulus, the ultimate threshold reached is normally not equal to 1.35 times the concentration of the adapting stimulus. For an adaptation concentration of 30 times the absolute threshold concentration, the ultimate threshold reached is about 1.1 the concentration of the stimulus used for the adaptation.

For d-octanol it is possible now to combine all the results

found for the raising of the threshold D for various concentrations C of the adapting stimulus in the formula:

$$D = 1 + a\ (C - 1)\ (1 - e^{-t/15\sqrt{C-1}}) \qquad (8)$$

where a varies only slightly for different concentrations of the adapting stimulus (e.g. from 1.10 to 1.35 for concentrations of the adapting stimulus of 30 to 100 times the absolute threshold concentration).

We have no intention to emphasize the formulae given in this section. The formula is only a mathematical expression which describes the course of the curves; it has not yet a physiological meaning.

III. When substituting in above formula D for C (the raised threshold equal to the stimulus concentration used for the adaptation) it is possible to give the A.T.C.S. (the adaptation time required for the cessation of the smell sensation) as a function of the adapting concentration. By assuming a value of 1.35 for a, the following relation for the A.T.C.S. is found

$$t = 20\ \sqrt{C - 1}\ .$$

The experimental results for d-octanol, plotted in figure 16 correspond with this relation.

IV. We shall discuss now the results plotted in figure 22, which give the relation between the raising of the threshold and the total quantity of odorous substance used for the adaptation (for different concentrations of the adapting stimulus).

When assuming small adaptation times and normal concentrations of the adapting stimulus, an approximation of the formula (8) is $D = 1/15\ .\ at\ \sqrt{C - 1}$. It is clear from this relation that the increased threshold concentrations for a fixed quantity of odorous substance supplied to the sense organ are inversily proportional to the square root of the concentration of the adapting stimulus. So for small adaptation times, and small quantities of odorous substance therefore, the raising of the threshold for a fixed quantity is largest when small concentrations of the adapting stimulus are used. This is in accordance with the experimental results, plotted in figure 22.

For long adaptation times, ultimately a constant threshold is reached which is about a factor 1.10 - 1.35 larger than the concentration of the adapting stimulus for the range of stimulus intensities investigated. So for large adaptation times, the in-

creased thresholds are nearly proportional to the stimulus concentrations used for the adaptation.

In figure 22, the curves are approaching this area of constant threshold values. As the curves intersect, the threshold concentrations do not differ much for the investigated area. A good approximation of the raising of the threshold as a function of the quantity of odorous substance supplied to the sense organ is therefore given by the curve plotted in figure 23.

V. It is possible to consider the formula for the threshold as a solution of the differential equation

$$\frac{dD}{dt} + \frac{D - 1}{15\sqrt{C - 1}} - \frac{a}{15}\sqrt{C - 1} = 0 .$$

It is clear from this equation that the rate at which the threshold is raised depends on the threshold already reached. In the beginning this rate is proportional to $\sqrt{C - 1}$ (C is the concentration of the adapting stimulus), later on the speed becomes smaller.

It is not our intention to emphasize the formula and the equation used for the raising of the threshold, neither to find a mechanism which can explain the differential equation.

A mechanism can only be given if much more results of adaptation investigations are available.

VI. As for the process of recovery it is not possible to express the results in one formula.

VII. The formulae and equations set up are of course approximations. As can be seen from figure 30, the adaptation process is mainly situated in the central parts of the sensory system. Yet the behaviour of the peripheral mechanism is also included in the equations, since the signals received by the central parts have not to be proportional to the adapting concentration C. So the equations give a combination of the adapting mechanism, and the transforming properties of the peripheral parts.

4.8. *A comparison of the adaptation phenomena for the sense of taste and smell*

Abrahams, Krakauer and Dallenbach (35) showed that the times between onset of the stimulus and the total cessation of the gustatory sensation is a function of the adapting concentration. The

adapting concentration was varied a factor 5 maximally. The curves showing the relationship between adapting concentration and adaptation time, required for a cessation of the gustatory censation, are analogous to the curves found for the sense of smell (see figure 16).

Hahn (36) performed various experiments to study the course of the adaptation process. The curves representing the relationship between the raising of the gustatory threshold and adaptation time prove to be analogous to the curves found for the sense of smell plotted in 4.5 and 4.6. Also the course of the recovery curves is the same for both sense organs.

Another important variable for gustatory adaptation is temperature. There appears to be a large influence of temperature upon the rapidity of adaptation. This effect is not surprising, because temperature also influences the absolute threshold. Yet there are discrepancies for some substances, e.g. glycocol. The absolute sensitivity of glycocol does not depend at all on temperature, but the rate at which adaptation proceeds is definitely a function of temperature.

For the sense of smell we concluded that the adaptation processes occur mainly in the central parts. The gustatory experiments conducted by Hahn furnish some evidence that for the sense of taste the adaptation may have, at least for a few substances, a peripheral rather than a central locus. Yet the analogous results of adaptation and recovery experiments for the sense of smell and taste suggest that perhaps also for the latter the central parts are important at adaptation processes.

4.9. *Survey of the main results of our investigations concerning adaptation processes*

1. Adaptation processes for the sense of smell are situated mainly in the central parts.
2. After previous adaptation, the curves representing the threshold concentration as a function of the time of recovery consists of two parts; at first the recovery is rapid, later on the recovery is much slower. The fast component is caused by the recovery of the central parts, the slow component very probably by the slow recovery of the peripheral parts.
3. If adaptation is ceased when the smell is no more perceived, the time of recovery, necessary to obtain a threshold twice as

low as the threshold when adaptation is stopped, is 40 seconds for d-octanol for all quantities of odorous substance used.

4. The thresholds after about 100 seconds' recovery are mainly controlled by the total quantity of odorous substance used for the adaptation. The raising of the threshold is not proportional to the quantity of odorous material supplied to the sense organs.

5. The adaptation times required for the cessation of the smell comply with the relation $t = b\sqrt{C-1}$, where b is a constant and C the concentration of the odorant used for the adaptation. The concentration is expressed in such a manner that $C = 1$ agrees with the absolute threshold concentration. For d-octanol the constant b is about 20, for m-xylene about 30.

6. The raising of the threshold during adaptation can be represented by the formula $D = 1 + a\,(C-1)(1 - e^{-t/15\sqrt{C-1}})$, where a is a constant varying only slightly for different adapting intensities, C the adapting concentration, D the raised threshold concentration and t the adaptation time. Odorant: d-octanol.

7. When during adaptation a constant threshold has not yet been reached, the raising of the threshold is approximately a function of the total quantity of odorant supplied to the sense organ. When a constant threshold value has been reached, this approximation is certainly not admissable.

8. The rate at which the threshold increases is in the beginning proportional to the square root of the adapting concentration. Later on this rate becomes smaller.

9. The equations set up are approximations. They are a combination of the adaptation mechanism of the central parts and the transforming properties of the peripheral parts.

Chapter V

THE RELATIONSHIP BETWEEN ODOUR, THRESHOLD AND CHEMICAL STRUCTURE

5.1. *Introduction*

Up to this time, many trials have been made to find a correlation between chemical structure and odour. An extensive survey of these theories has been given by Moncrief (47) and Lauffer (48).

The classification of odours is difficult. The smell of an odorous substance even depends on the concentration of the odorant in the air inhaled. The smell of β Ionone for instance resembles the smell of cedar wood when strong concentrations are used, but has an odour of violets when diluted. A comparison of the smell of two different substances is only justified if the concentration of the odorant, with the threshold concentration as a unit, is recorded. This has never been done so far.

Adrian (49) analyzed the electrical activity of the mammalian olfactory bulb. He observed that different parts of the bulb were activated when different odorants were used. On the ground of the results obtained in the investigations of the olfactory bulb, Adrian comes to the conclusion that the discrimination of odours depends on the differences in the temporal and spatial pattern of excitation as well as on the differential sensitivity of the various receptor types. The hypothesis that the spatial distribution of the responses are due to a localised projection of the different areas of the olfactory epithelium on to the bulb is confirmed by histological studies by Le Gros Clark (50).

So the problem of finding a relationship between odour and chemical structure is a very complicated one, because probably the arrangement of the connections between the peripheral and central parts strongly determines the smell ultimately perceived.

The search for a relationship between threshold and chemical structure or some physical or chemical property looks more promising. Davies and Taylor (51) have found a close parallelism between the activity of a series of substances in accelerating haemolysis of red blood cells and their thresholds as odorous

substances. Perhaps there is some resemblance between the penetration and reaction of the accelerator with the cell membrane and the action of the odorants upon the membrane of the sense cell.

The more a given substance is concentrated in the lipid at the lipid-water interface of the receptor, the more efficient it will be. The work of Backman and Zwaardemaker (52) indicates that there is indeed a correlation for homological series between lipid-water partition coefficients and odorous thresholds.

5.2. *Threshold and optical isomerism*

It is well-known than optically active isomers, despite the fact that they are identical in structure, are different in physiological respects. A striking example of different physiological activity observed is vitamin C or ascorbid acid, the dextrorotary acid is active, the laevo-rotary form being physiologically inactive. Similar differences in physiological activity are associated with odour properties. Many investigators state that the *d* and *l* components have different odours. So far only a few thresholds have been determined. Doll and Bournot (53) determined the thresholds of the *d* and *l* component of menthol. They found the thresholds to be equal. Naves came to the same conclusion for *d* and *l* α Ionone (54).

According to Guillot and Babin (55) the threshold of the *d* component of 2-octanol (hexyl methyl carbineol) is 3.2 times lower than the threshold of the *l* component. To check this, the same experiment was repeated by us.

The optical isomers were separated according to the process of Kenyon (56). The boiling points of the *d* and *l* component respectively are 84 – 84.5°C (at 18 mm) and 85 – 86°C (at 19 mm). The refractive indexes are for both components 1.421 ± 0.001 at 20°C, the specific rotation is 9.75 degrees at 22°C for the *d* component and -9.81 degrees at 18°C for the *l* component.

At normal smelling a small difference in the odour of the components is already noticed. If threshold concentrations are used, it is more difficult to distinguish the *l* component from pure air than the *d* component. For normal inspiration (500 cc per second), the threshold concentrations of the *d* and *l* octanol were 5.2 and 1.5×10^9 molecules per cc respectively. The vapour pressure used for both substances is 8.10^{-2} mm of mercury; this value was

determined from the boiling points at the pressures of 76 and 1.8 cm of mercury in the same manner as discussed in chapter II.

So the threshold of *l* octanol was 2.9 times larger than the threshold of the *d* component, which is in good agreement with the value of 3.2 found by Guillot and Babin.

5.3. *Thresholds of ortho-, meta- and para-compounds*

The threshold concentrations of a number of ortho-, meta- and para-compounds have been determined for normal inspiration with the author as subject. The same determinations were performed for the xylenols.

The results of the experiments are given in table 3. The apparatus used was discussed in Chapter I, the vapour pressures of the substances (at 0°C) were obtained by interpolation or extrapolation of the vapour pressure data given in Jordan (9).

Some of the odorants examined showed strong adsorption to glass surfaces. The influence upon threshold experiments is discussed in chapter I.

The adsorption appeared to be strong for 1 : 3 – xylen – 2 – ol, 1 : 4 – xylen – 2 ol, 1 : 2 – xylen – 3 ol and 1 : 3 – xylen – 5 ol. No influence of the adsorption during threshold experiments was perceptible for the ortho-, meta- and para-compounds of toluidine, nitrophenol and xylene.

Table 3

Substance	Threshold	Substance	Threshold
o-xylene	$1.2\ 10^{13}$	o-cresol	$2.4\ 10^{9}$
m-xylene	$2.2\ 10^{12}$	m-cresol	$2.0\ 10^{9}$
p-xylene	$3.2\ 10^{11}$	p-cresol	$2.8\ 10^{8}$
o-toluidine	$6.4\ 10^{11}$	o-nitrophenol	$5.0\ 10^{9}$
m-toluidine	$1.1\ 10^{13}$	m-nitrophenol	$1.3\ 10^{13}$
p-toluidine	$6.6\ 10^{11}$	p-nitrophenol	$1.0\ 10^{13}$
1:3-xylen-2-ol	$8.0\ 10^{8}$	1:3-xylen-5-ol	$2.0\ 10^{8}$
1:4-xylen-2-ol	$2.6\ 10^{9}$	1:3-xylen-4-ol	$2.6\ 10^{9}$
1:2-xylen-3-ol	$2.6\ 10^{9}$	1:2-xylen-4-ol	$1.7\ 10^{10}$

The table can be supplied with thresholds already known (see e.g. (57)).

Substance	Threshold	Substance	Threshold
benzene	$4\ 10^{13}$	phenol	$1\ 10^{13}$
toluene	$1.3\ 10^{13}$	nitrobenzene	$1\ 10^{12}$
aniline	$5\ 10^{13}$	pseudocumene	$1\ 10^{12}$
durol	$4\ 10^{11}$	cumidine	$1\ 10^{12}$

The thresholds are given in molecules per cc.

Some rules on the changing of the threshold with chemical structure can be deduced from the threshold values given in the table. Starting with benzene, the threshold changes little when a methyl or hydroxyl group is attached to the benzene ring. If the attached group is a nitro- or amine-group, the threshold decreases with a factor 400 and 10 respectively.

If 2 methyl-groups are attached, the threshold decreases about a factor 10. If two hydroxyl-groups are attached to the ring, the threshold increases strongly (resorcinol, hydroquinone and catechol are odorless, according to Moncrief (47)).

If however a combination of a methyl and hydroxyl group is attached to the ring, the threshold decreases about a factor 10^4. An addition of a second methyl group (xylenols) has little influence upon the threshold.

A second property for substances with at least one methyl group lies in the fact that the symmetrical molecules have the lowest threshold among the corresponding substances. The para-compounds of xylene, cresol and toluidine have the lowest threshold in their series, while from the xylenols 1 : 3 – xylen – 5 – ol and 1 : 3 – xylen – 2 – ol have the lowest threshold.

The behaviour of nitrophenol shows a deviation, here the ortho-compound has the lowest threshold.

We did not succeed in finding a correlation between the thresholds of the ortho-, meta- and para-compounds and some physical or chemical properties such a solubility, boiling point and melting point, number of possible valence-bond structures, dipolmoments of the group attached to the ring or surface tension lowering in water *).

*) The author is much indebted to H.Postma, J.R.Nooi, E.J.Stamhuis and C.H.Geerts for their assistance during the experiments described in this thesis and to R.J.Planta for preparing the optical isomers and a number of mercaptans.

SUMMARY

The biophysical investigations described in this thesis are related to the mechanism of the sense of smell. Various aspects, such as the absolute threshold, the number of co-operating receptors required for a threshold sensation, adaptation and recovery of the sense organ and the connection between chemical structure and threshold were investigated.

The apparatus designed for the experiments and the requirements to be fulfilled were discussed in chapter I.

For the investigation of the absolute threshold it is important to determine the most favourable values for the changeable quantities. Variation of the stimulus duration and the rate of flow of the inspired or injected air influences the threshold to a large extent. Below a critical duration of about 0.18 seconds, the minimum number of molecules required for a smell sensatio· approaches a constant value. When changing the stimulus duration from for instance 0.1 second into 0.05 second, about an equal number of molecules is required for a threshold sensation, so the minimum concentration of the odorant shows a nearly twofold increase. Above the critical time the minimum concentration remains almost independent of the stimulus duration, here the total number of molecules is about proportional to stimulus duration.

The variation of the threshold with the rate of flow of the inspired or injected air can be explained by taking into account the diffusion and absorption phenomena in the nasal cavity. Two opposed effects are operating when the rate of flow increases, the concentration of the odorant entering the olfactory slit increases because less molecules are lost in the mucous membrane before the slit is reached, but the fraction of the molecules diffusing to the olfactory epithelium decreases. The influence of the two effects upon the threshold was discussed in chapter III. For the calculations, the fraction of the air passing the olfactory slit should be determined. Experiments with a model showed that this fraction was about 7 percent for normal inspiration. When using small volumes for the threshold determination a correction for the lost volume in the foremost part of the nasal cavity also has to be applied.

In chapter III it was pointed out that concentration of the odorous molecules from a large area of the olfactory epithelium upon one sense cell is not possible. Nine molecules of the most sensitive mercaptans are certainly sufficient to activate a sense cell. Very probably even one molecule is already sufficient.

From the influence of the statistical fluctuations in the number of molecules striking the sense organ upon the frequency of smelling it can be concluded that for the most sensitive mercaptans at least 40 receptors must be activated simultaneously before a sensation is perceived. The efficiency of the smell mechanism was discussed.

Various phenomena concerning the adaptation and recovery of the sense organ were discussed in chapter IV. Adapting intensity and adaptation time appear to be important quantities. Adaptation during a time t to a concentration C of the odorant ($C = 1$ agrees with the absolute threshold concentration) gives a raising of the threshold D expressed for d-octanol in the formula

$$D = 1 + a\,(C - 1)\,(1 - e^{-t/15\sqrt{C-1}}) ,$$

where a is a constant varying only slightly with the adapting concentration.

The adaptation times required for a cessation of the smell sensation are proportional to the square root of the concentration used for the adaptation. If adaptation is not yet complete, the raising of the threshold is approximately a function of the total quantity of odorous material supplied to the sense organ, irrespective of the time in which this quantity has been supplied.

Some experiments concerning the recovery of the sense organ after previous adaptation indicated that the peripheral parts of the sense organ contribute only to a small extent to the adaptation. An extensive survey of the results of adaptation investigations was given in 4.9.

A problem still unsolved is the relationship between chemical structure or some chemical or physical property, and odour or threshold. A number of thresholds of ortho-, meta- and para-compounds and two optical isomers was determined. Even for the limited group of materials investigated, no correlation was found between threshold and some chemical or physical property. (Chapter V).

S A M E N V A T T I N G

De in dit proefschrift beschreven biofysische onderzoekingen hebben betrekking op het mechanisme van het reukorgaan. Verschillende aspecten zoals de absolute gevoeligheid, het aantal samenwerkende receptoren bij een drempel-sensatie, adaptatie en herstel van het orgaan en het verband tussen chemische structuur en drempelwaarde werden onderzocht.

De voor deze onderzoekingen ontwikkelde apparatuur en de eisen, welke men hieraan moet stellen, zijn in het eerste hoofdstuk besproken.

Voor de bepaling van de absolute gevoeligheid van het orgaan is het van belang om de meest gunstige waarden voor de verschillende parameters te kiezen. De prikkelduur en de stroomsnelheid van de ingeademde of geinjecteerde lucht beinvloeden de drempel sterk. Beneden een bepaalde kritische tijd (ongeveer 0.18 seconde) blijkt het minimale aantal reukstofmoleculen, welke nodig is voor een sensatie tot een constante waarde te naderen. Verandert men bijvoorbeeld de prikkelduur van 0.1 seconde in 0.05 seconde dan zijn ongeveer evenveel moleculen nodig en zal dus de minimale concentratie ongeveer twee maal zo groot worden. Boven de kritische tijd heeft een verandering van de prikkelduur weinig invloed op de minimale concentratie. Het totale aantal moleculen nodig voor een drempelsensatie is dan ongeveer evenredig met de prikkelduur (hoofdstuk II).

Het verband tussen drempel en stroomsnelheid van de geinjecteerde of ingeademde lucht kan worden verklaard door de absorptie en diffusie verschijnselen in de neusholte in rekening te brengen. Hierbij heeft men twee tegengestelde effecten; wanneer de stroomsnelheid toeneemt wordt de concentratie van de reukstof in de reukspleet groter omdat een kleiner percentage moleculen verloren gaat voordat de reukspleet wordt bereikt; echter het percentage van de moleculen, welke gelegenheid krijgt om naar het reukepitheel te diffunderen vermindert. In hoofdstuk III is de invloed van deze twee effecten op de drempel voor verschillende stroomsnelheden berekend en vergeleken met de experimentele waarden. Voor de berekening moet men weten welk percentage van de ingeademde of geinjecteerde lucht de reukspleet passeert. Door

middel van enkele stromingsproeven met een model van de neusholte werd dit percentage bepaald. Indien bij de drempelwaarde metingen kleine volumina worden gebruikt (hoge concentraties) moet een correctie worden aangebracht voor het volume, welke in het voorste deel van de neusholte verloren gaat.

In hoofdstuk III is tevens aangetoond, dat concentratie van de reukstof uit een groot gebied van het reuk-epitheel op 1 zintuigcel niet mogelijk is. Voor de gevoeligste mercaptanen blijken 9 moleculen reeds voldoende te zijn om een zintuigcel te activeren. Zeer waarschijnlijk is echter ook 1 molecuul van de reukstof reeds voldoende.

Uit de invloed van de statistische fluctuaties in het aantal moleculen, welke op het zintuigorgaan vallen, op de reukkans kan men berekenen dat voor de gevoeligste mercaptanen, voor het ontstaan van een reuksensatie, minstens een 40-tal receptoren gelijktijdig moet worden geactiveerd. Indirect komt hierbij de efficiency van het reukmechanisme ter sprake.

Een aantal adaptatie verschijnselen is in hoofdstuk IV besproken. De sterkte van de prikkel en de adaptatie tijd blijken belangrijke variabelen te zijn. Adaptatie gedurende een tijd t aan een reukstofconcentratie C (C = 1, komt overeen met de absolute drempelconcentratie) geeft voor d-octanol een drempelverhoging D, welke wordt gegeven door de formule

$$D = 1 + a\,(C - 1)\,(1 - e^{-t/15\sqrt{C-1}}) ,$$

waarbij a een constante is, die maar weinig van de reukstof concentratie C afhangt.

De adaptatie tijden, welke voor een bepaalde reukstofconcentratie nodig zijn om de geur te doen verdwijnen blijken evenredig met de wortel uit de concentratie te zijn. Tevens is aangetoond, dat indien de adaptatie nog niet volledig is, de verhoging van de drempel bij benadering wordt bepaald door de totale hoeveelheid reukstof, welke aan het orgaan wordt toegevoerd, onafhankelijk van de tijd, waarin deze hoeveelheid wordt aangeboden.

Enkele proeven over het herstel van het reukzintuig tonen aan, dat de adaptatie slechts voor een gering gedeelte wordt veroorzaakt door het perifere gedeelte van het orgaan. Het centrale zenuwstelsel blijkt zeer sterk tot de adaptatie bij te dragen. Een uitgebreid overzicht van de resultaten van de adaptatie metingen is gegeven in 4.9.

Een onopgelost probleem blijft de relatie, welke tussen de geur of drempelwaarde en de chemische structuur of een of andere

chemische of physische eigenschap bestaat. Een aantal drempelwaarden van ortho-, meta- en para-stoffen en een tweetal stereo isomeren werden bepaald. Zelfs voor de beperkte groep onderzochte stoffen kon geen correlatie met een chemische of physische eigenschap worden gevonden (hoofdstuk V).

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