ELECTRON MICROSCOPY OF NORMAL AND REGENERATING OLFACTORY EPITHELIUM IN MAN AND THE CAT

H. J. C. VAN SCHERPENBERG

ELECTRON MICROSCOPY OF NORMAL AND REGENERATING OLFACTORY EPITHELIUM IN MAN AND THE CAT

ELECTRON MICROSCOPY OF NORMAL AND REGENERATING OLFACTORY EPITHELIUM IN MAN AND THE CAT

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE LEIDEN OP GEZAG VAN DE RECTOR MAGNIFICUS DR. S. E. DE JONGH, HOOGLERAAR IN DE FACULTEIT DER GENEESKUNDE, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP WOENSDAG 12 MAART 1958 TE 15 UUR

DOOR

HENRI JEAN CORNEILLE VAN SCHERPENBERG
geboren te Nijmegen in 1925

UNIVERSITAIRE PERS LEIDEN
LEIDEN 1958

PROMOTOR: PROFESSOR DR. H. A. E. VAN DISHOECK

Aan de nagedachtenis van mijn vader
Aan mijn moeder
Aan mijn vrouw

CURRICULUM VITAE

Op 31 juli 1925 werd H. J. C. VAN SCHERPENBERG geboren te Nijmegen. Hij bezocht het Nijmeegs Stedelijk Gymnasium en behaalde in juni 1944 het einddiploma.

Door oorlogsomstandigheden en wegvoering naar Duitsland kon hij zijn medische studie eerst in september 1945 beginnen. Hij werd ingeschreven als student aan de Medische Faculteit van de Rijksuniversiteit te Leiden, daar zijn vader ook aan deze Universiteit gestudeerd had. Hij werd candidaat in de medicijnen in october 1947 en doctorandus in juni 1950.

In augustus 1950 begon hij zijn werkzaamheden aan de afdeling keel-, neus- en oorheelkunde van het Academisch Ziekenhuis te Leiden, destijds nog onder leiding van Prof. Dr P. H. G. VAN GILSE als student-assistent, terwijl hij in october 1952 assistent buiten bezwaar van 's lands schatkist van deze afdeling werd.

Februari 1953 was de maand waarin de promovendus in de artsenstand werd opgenomen. Helaas was hij in maart 1953 verplicht zich in militaire dienst te begeven. Hij was in die tijd werkzaam bij de Militair Geneeskundige Dienst te Utrecht en gedurende 4 maanden op de keel-, neus- en oorafdeling van het Centraal Militair Hospitaal aan de Springweg te Utrecht.

Hij begon zijn opleiding tot keel-, neus- en oorarts op 1 october 1954 aan de afdeling keel-, neus- en oorheelkunde van het Academisch Ziekenhuis te Leiden. Op 17 januari 1958 werd hij hoofd-assistent van die afdeling.

Dit proefschrift werd bewerkt in de afdeling keel-, neus- en oorheelkunde van het Academisch Ziekenhuis te Leiden.

De onderzoekingen werden verricht met medewerking van leden van het Histologisch Laboratorium, speciaal de afdeling Electronen Microscopie.

CONTENTS

SAMENVATTING EN ALGEMENE BESCHOUWING

De vele onopgeloste problemen betreffende het normale en pathologische reukepitheel, brachten ons ertoe om nieuwe gegevens met behulp van de electronen microscoop te verzamelen. Daarbij trokken de reukvesikels, de haarvormige structuren op het reukepitheel, de membrana limitans olfactoria van VON BRUNN, het pigment en de regeneratie van het epitheel onze aandacht. Bij electronen microscopisch onderzoek van het normale reukepitheel in de kat vonden wij verschillende typen van reukepitheel, vooral verschillend wat betreft hun oppervlaktestructuren, als ook de rangschikking van hun kernen.

Bij het algemeen beschreven en bekende type, hier type I genoemd, liggen de vesiculae olfactoriae boven het celoppervlak. De protoplasmalaag zonder kernen wordt naar onderen begrensd door een laag steuncelkernen, die weer begrensd is door de donker gekleurde osmiophiele reukcelkernen. Basaalcellen zijn bij het volledig ontwikkelde epitheel sporadisch te vinden.

Bij type II worden de vesiculae olfactoriae in de dalen — gevormd door de wanden der steuncellen — gevonden. De bovenste rij kernen zijn hier reukcelkernen, waaronder steuncelkernen liggen, in tegenstelling tot type I. Onder de rij steunkernen ligt een enorme hoeveelheid basaalcellen, die de mogelijkheid van regeneratie doet vermoeden. In deel II van dit werk wordt dan ook de conclusie getrokken, dat dit een regeneratiestadium van het reukepitheel is.

Het bestaan van de membrana olfactoria limitans van VON BRUNN wordt bevestigd door het vinden van reukepitheel type III. In dit epitheel is een groot netwerk van tussenschotten tussen de steun- en reukcellen aanwezig. Deze tussenschotten omgeven grotendeels de reukcellen en de uitvoergangen van de klieren van Bowman. Dicht onder het oppervlak smelten ze samen tot een syncytiale cuticula bestaande uit de meest perifere delen der steuncellen; deze cuticula is op sommige plaatsen dik, op andere plaatsen dun of afwezig. Hij is doorboord door de perifere uitsteeksels van de reukcellen. Op de oppervlakte van de cuticula ziet men knotsvormige of bloemkoolachtige aanzwellingen, waarvan tubulaire protoplasma-uitsteeksels uitgaan; deze laatsten worden ook op de steuncel aangetroffen. Tussen deze aanzwellingen liggen de vesikels. Deze membraan en de tussenschotten bestaan uit een dichte, schijnbaar amorphe, osmiophiele substantie, waarin donkere granula gezien kunnen worden; deze structuren worden verondersteld een intercellulair of een intracellulair product van de steuncellen te zijn.

Dit type III wordt steeds in het achterste deel van de neusholte van drie katten

1

gevonden. De topografische projectie van dit gebied op het achterste deel der bulbus, aangetoond door LE GROS CLARK en WARWICK, en het grote aantal actiepotentialen in dit deel der bulbus opgewekt bij ruiken van vlees en vis of vluchtige oliën, (ADRIAN 1956) maken het waarschijnlijk, dat dit type overwegend gevoelig is voor deze stoffen.

Het perifere deel van het reukneuron bevat veel draadvormige donkere structuren, die uit conglomeraties van protofibrillen schijnen te bestaan; deze structuren ontbreken in het meest perifere gedeelte vlak onder en in de vesicula olfactoria. In de vesiculae zijn geen holten, wel kleine vacuolen. Vanaf de vesikel ontspruiten minstens 36—40 reukharen, die uitgaan van een basaallichaam of diplosoom. De basale dwarsplaten van deze basaallichamen zijn onderling door electronen-dichte lijnvormige structuren verbonden. De cilia lopen — althans in de gemaakte preparaten — meest evenwijdig aan de oppervlakte. Als de vesikel in een dal ligt, lopen de cilia olfactoria eerst loodrecht omhoog, waarna ze, als ze boven de oppervlakte uitkomen, hieraan evenwijdig gaan lopen. Zij rusten op de tubulaire uitsteeksels der steuncellen. De cilia olfactoria hebben eenzelfde bouw als alle trilharen gevonden in de hele fauna en flora: een axiale fibrillenbundel bestaande uit een buitenste laag van negen dubbele fibrillen en twee fibrillen in het centrum en dit geheel omgeven door een schede van een granulaire structuur. De schede gaat over in de bedekkende cuticulaire protoplasmalaag op de vesikel. De fibrillenbundel is ook terug te vinden in het basaallichaam; hij gaat door de basale dwarsplaat en vervolgt zijn weg door de vesikel om daar grotere bundels fibrillen te vormen, die eindigen tussen of in de draadvormige structuren.

De steuncel is op dwarsdoorsnede zeshoekig; de reukceldendrieten liggen ingedrukt in de wanden van de steuncellen. In overeenstemming met GASSER worden ook door ons in de steuncel circulair en longitudinaal lopende structuren van het endoplasmatisch reticulum gevonden. In de steuncel worden oppervlakkig ook secretieproducten gezien. Wij opperen, dat de secretieholten zich mogelijk ontledigen via kleine openingen in het cuticulaire celoppervlak of via de tubulaire protoplasma-uitsteeksels. De steuncel vertakt zich naar onderen in uitlopers van allerlei vormen. Deze vertakkingen zijn vaak gevuld met pigmentgranula en een dichte massa osmiophiele, korrelige protoplasmastructuren in verbinding staande met de tussenschotten der membrana olfactoria. In reukepitheel van jonge katten en mensen en ook in pathologisch en regenererend reukepitheel worden zeer weinig pigment granula gevonden. Bij het volledig ontwikkelde reukepitheel van de kat wordt het pigment meest basaal in rijtjes in de vertakkingen van de steuncellen en subepitheliaal in de klieren van Bowman gevonden. Bij de mens worden door de hele steuncel heen de granula aangetroffen en ook in de klieren van Bowman. Zowel in de klieren van Bowman als in de steuncellen kunnen deze granula kleiner worden, doordat ze vervloeien en zo secreet vormen.

In overeenstemming met de onderzoekingen in vivo van SCHULTZE en HOPKINS worden door ons bij mens en kat met de electronen microscoop twee soorten haarvormige structuren op het epitheel gevonden. Deze onderzoekers vermelden echter in vivo een grotere lengte van de haren. Vermoedelijk zijn de „haren" door de kunstbewerkingen afgebroken of opgelost, dan wel omgebogen, zodat in de preparaten de

ware lengte niet vastgesteld kan worden. De tubulaire protoplasma-uitsteeksels op de steuncellen, aan de basis breder en perifeer steeds dunner wordend, vormen een plexus. Zij zijn niet identiek met microvilli. Het secreet wordt in dit netwerk vastgehouden. Voorts vormt het een skelet, waarop de vesikels en de cilia olfactoria rusten.

In een basaalcel worden soms groepen van axonen gevonden, elke groep omgeven door membranen, die volgens GASSER gevormd schijnen te worden door zeer diepe invaginaties van de protoplasmamembraan. Deze protoplasmamembraan gaat subepitheliaal geleidelijk over in de celmembraan van de Schwannse cellen. Dit is ook in overeenstemming met onze bevindingen.

Bij de volwassen kat en mens zien wij groepen van kliercellen van Bowman in verschillende secretiestadia. Deze worden aangetroffen in niet scherp gescheiden lagen. Het diepste liggen de indifferente cellen in het zogenaamde depôtstadium. Deze indifferente cellen, die practisch geen granula en weinig mitochondriën bevatten, worden bij jonge katten en baby's gevonden en zijn speciaal bij pathologisch en regenererend reukepitheel duidelijker zichtbaar. Hierboven en ook voor een klein gedeelte er tussen liggen vooral in volledig ontwikkeld reukepitheel de praesecretoire en actieve secretoire kliercellen. De praesecretoire kliercellen zijn vol met secretoire granula. Bij de secretoire kliercellen worden deze granula door vervloeiing, beginnend aan de periferie der korrels, in secreet omgezet. Het oppervlak van de kliercellen, de intercellulaire kanaaltjes en de klierbuizen zijn bedekt met kleine, haarvormige, tubulaire uitsteeksels, korter en kleiner in diameter dan die van de steuncellen. De secreetlaag, die op deze structuren ligt, suggereert een mogelijke functie van deze uitsteeksels bij de secretie. De secreetvorming schijnt te beginnen in het apicale deel der cel, en later pas basaal in de cel.

Nog hoger wordt het stadium gezien waarin de cellen van de klier van Bowman practisch geheel in rust zijn na de secreetvorming. De kleine holten zonder secreet in deze cellen zijn omgeven door kleine puntvormige granula, welke waarschijnlijk resten van de pigmentgranula zijn. De kernen, in tegenstelling tot de celkernen in de andere stadia, zijn door hun verschillende osmiophilie donker en minder donker gekleurd. Celgroepen in dit stadium reiken soms tot in het epitheel. Intra-epitheliaal boven de basaallaag is de klierbuis niet meer omgeven door kliercellen, maar door reukcellen en steuncellen, die in epitheel type III verbonden zijn met het netwerk van tussenschotten uitgaande van de membrana olfactoria. Zo blijkt, dat de kliercellen met de reuk- en steuncellen één systeem vormen. Dit maakt een mogelijke overgang van de kliercellen via de basaalcellen in de reuk- en steuncellen aannemelijker.

Het verband tussen de grotere gevoeligheid en het minder snel uitgeput zijn van reukepitheel met een grotere hoeveelheid pigment, gevonden door ADRIAN, en ook de afwezigheid of sterke vermindering van reuk bij albino's, gevonden door HUTCHINSON en OGLE, wijst op het grote belang van het pigment bij het reukproces. Het blijkt, dat de pigmentgranula in de steuncellen en kliercellen kunnen vervloeien tot secreet. In de klieren van Bowman zijn zij actief secretoir, maar in de steuncellen en de basaalcellen meestal in rust. Een grote hoeveelheid pigment in een bepaald gedeelte van het reukepitheel wijst dus op de mogelijkheid om er meer secreet te produceren, dan in

andere gedeelten. Deze observaties maken het waarschijnlijk dat het secreet van de klieren van Bowman bij kat en mens onontbeerlijk is bij het ruiken.

Van de verschillende mogelijke pathologische veranderingen van het reukepitheel bestudeerden wij speciaal de resultaten van chemische beschadigingen. Voorts waren in een deel van ons materiaal infectieuze laesies aanwezig. In de literatuur wordt vaak een overgangsvorm naar trilhaarepitheel beschreven. Bij grote vergrotingen echter observeren wij, dat de cilia van een kleinere lengte en diameter zijn dan die van het respiratorische epitheel. Wij willen deze cilia dus microcilia noemen. De vesiculae olfactoriae zijn afwezig en de tubulaire protoplasma-uitlopers zijn verkort. De microcilia zouden mogelijk kunnen ontstaan uit de praeciliaire tubulaire protoplasma-uitsteeksels der steuncellen. Onder zo'n uitsteeksel verschijnt een diplosoom, waaruit waarschijnlijk een fibrillenbundel groeit in de as van dit buisje, zodat de protoplasma-uitloper tot schede van de fibrillenbundel van een microcilium kan worden. Bij chronische infectie groeien deze microcilia uit tot echte cilia, zodat dit gebied bedekt met cilia op het respiratorisch epitheel gelijkt. Evenwel kunnen subepitheliaal de klieren van Bowman en de zenuwbundels toch jarenlang blijven bestaan. De microcilia fungeren mogelijk als verdedigingsmechanisme tijdens een neusinfectie.

De basaalcellen worden dikwijls vervangingscellen genoemd. Het is echter volgens sommige auteurs onmogelijk om deze onrijpe cellen te differentiëren met zenuwweefsel kleurmethoden, zoals wij ook ondervonden. Volgens NAGAHARA (1940) groeit bij de muis en volgens SCHULTZ (1941) bij de apen de centrale uitloper van de regenererende reukcellen het eerst uit. 43 dagen na de kunstmatig opgewekte regeneratie vinden wij in het reukepitheel van de kat een syncytiale laag cellen voorzien van langgerekte osmiophiele kernen en basaal centrale uitlopers, protofibrillen bevattend. Sommige cellen bevatten ronde, matig osmiophiele kernen en secretieresten. Na 46 dagen van regeneratie zijn de toekomstige reukcellen met hun vesiculae olfactoriae in statu nascendi zichtbaar. Deze cellen bevatten osmiophiele kernen en zijn voorzien van centrale uitlopers. In normaal reukepitheel bestaat ook een verschillende graad van osmiophilie van reuk- en steuncelkernen. Het lijkt waarschijnlijk, dat de basaalcellen, die osmiophiele kernen en centrale uitlopers bevatten, de toekomstige reukcellen zijn en de cellen, die secretieresten en matig osmiophiele kernen bevatten de toekomstige steuncellen zijn. Dezelfde beelden worden gevonden bij het menselijk reukepitheel.

43 dagen na de kunstmatig opgewekte regeneratie zijn bij de kat in de basale syncytiale laag cellen met secreetresten zichtbaar. Deze resten nemen in hoeveelheid af in de cellen, die verder van de hals der klierbuis afliggen. De indifferente kliercellen liggen voor het merendeel in de diepste lagen van het sub-epitheliale weefsel en het secreet wordt geleidelijk gevormd in de hoger gelegen cellen. Deze basaalcellen zijn dus waarschijnlijk rustende kliercellen en kunnen hun oorsprong hebben uit de meer secreet bevattende kliercellen om de buis. Aangezien de subepitheliaal gelegen klierbuizen in het epitheel

dringen — bij beiden ontbreekt een membrana propria — en de intra-epitheliale uitvoergangen omgeven worden door reuk- en steuncellen, is het aannemelijk, dat de kliercellen van onderen af overgaan in de basale laag. Embryologisch ontstaan de klier- en basale cellen gelijktijdig uit het neurectodermale embryonale reukepitheel.

Wegens de waarschijnlijke verplaatsing van de rustende kliercellen van Bowman naar de laag van de basaalcellen, wordt verondersteld, dat de kliercellen, die een zeer osmiophiele kern en soms een osmiophiel uitsteeksel bezitten, de voorlopers van de reukcellen zijn. De kliercellen, die matig osmiophiele kernen, kleine pigmentgranula en secretieresten bezitten, zijn vermoedelijk de voorlopers van de steuncellen. Dit is alles overeenkomstig met de verschillende graad in osmiophilie van de steun- en reukcelkernen in normaal reukepitheel. Men kan zich verwonderen, dat sensorische cellen ontstaan uit kliercellen, maar het embryonale karakter van normaal en regenererend reukepitheel maakt dit aannemelijk. Berger (1926) beschreef in het esthesio-neuro-epithelioom rosetten van cellen gelijkend op steuncellen en bipolaire reukcellen liggend om een buis.

Er wordt verondersteld, dat de onrijpe steuncellen zich in de basaallaag delen. De toekomstige reukcellen zijn reeds zo hoog gedifferentieerd wegens de aanwezigheid van protofibrillen in hun centrale uitlopers in de basaallaag, dat zij zich niet meer kunnen delen. Bij herhaalde regeneratie na recidiverende heftige infecties met verwoesting van de reukcellen, zal de voorraad van kliercellen van Bowman uitgeput raken, wegens het vermoedelijk ontbrekende of afnemende vermogen tot deling; hierdoor zal de regio olfactoria afnemen in omvang. Dit kan de oorzaak van de afname der reukzin bij oudere personen zijn, gevonden door Versteeg (1956). Smith (1937) vond een gedeeltelijk of totaal verlies van zenuwvezels in de bulbus olfactorius van oudere personen, wat op hetzelfde feit wijst.

Een duidelijke analogie vinden wij tussen de embryologische ontwikkeling van het reukepitheel en de regeneratie, vooral van de oppervlaktestructuren. Embryonaal verschijnt ook eerst het centrale uitsteeksel van de reukneuroblast, pas later ontwikkelt zich de perifere uitloper met vesikel. Van der Stricht beschreef reeds bij dwarsdoorsneden van het epitheel grotere en kleinere polygonale reukgebieden tijdens de embryonale ontwikkeling. Uit de kleinere polygonen, die al kleiner en ronder worden, ontstaan de sensorische cellen. Er bevinden zich in de reukvelden in statu nascendi centrosomen, die zich verdelen in centriolen en naar de periferie bewegen en zo worden tot diplosomen waaruit de cilia olfactoria ontspruiten. Dit zien wij eveneens bij de regeneratie van het oppervlak van het reukepitheel van de mens na een infectie en bij de kunstmatig opgewekte regeneratie van het reukepitheel van de kat. Aanvankelijk liggen de kernen tijdens de embryonale ontwikkeling en bij de regeneratie vlak onder het oppervlak. De osmiophiele reukcelkern met de centrioolachtige structuren lijken op deze manier het oppervlak tot de vorming van een vesicula olfactoria te induceren. Hierna schijnen de reukkernen met het grootste deel van het protoplasma vrij snel van uit hun heuveltjes naar de diepere lagen te migreren. Hierbij worden de perifere uitlopers bij het oppervlak kleiner en steeds smaller en tenslotte komen de vesiculae olfactoriae in statu nascendi diep in het dal tussen de aangrenzende aanstaande steuncelgebieden te liggen. Later kunnen de perifere

uitlopers langzaam uitgroeien en kunnen de reukvesikels meer op het niveau van het toekomstige steuncelloppervlak komen te liggen. De steuncellen schijnen zich te begeven naar de bovenste laag, terwijl ze met hun perikaryon en Golginetwerk tijdens hun migratie de reukkern indeuken. Er bestaat zo in het syncytium een tegengesteld gerichte migratie van de reukkernen en steunkernen.

Reukepitheel type II van de kat met de vesikels in dalen en een syncytium van de bovenste lagen schijnt een regeneratiestadium te zijn. Er zijn dus bij de kat twee normale reukepitheeltypen aangetroffen: één zonder en één met de membrana olfactoria van VON BRUNN. Alle andere beschreven vormen zijn dus waarschijnlijk pathologische en regenererende vormen van het reukepitheel.

In pathologisch reukepitheel van de volwassen mens blijkt het, dat de reuk- en steuncellen niet noodzakelijk vernietigd behoeven te worden door een infectie. De oppervlaktestructuren zijn slechts beschadigd: de vesiculae olfactoriae zijn verdwenen en de tubulaire uitsteeksels zijn verkort. Onder de oppervlakte zijn verschillende centrioolachtige structuren zichtbaar. De eerste rij kernen onder het oppervlak zijn osmiophiele, langgerekte kernen, die aan hun basis zijn ingedeukt door of liggen tussen steuncellen. Deze reukcelkernen schijnen te migreren naar de oppervlakkige laag, waar zij mogelijk de groei van de uitstekende structuren induceren.

Wij zijn ons bewust van het feit, dat dit werk slechts een begin van een volledig onderzoek van de gehele pathologie van het reukorgaan vormt. Mag onze poging anderen er toe brengen om hiermee verder te gaan, teneinde het bestaande hiaat op te vullen.

CONCLUSIES:

1. Er zijn bij de kat twee reukepitheeltypen, één zonder en één met de membrana limitans olfactoria van VON BRUNN. Dit laatste type schijnt achter in de neus te zijn gelegen en is waarschijnlijk gevoelig voor de geur van vluchtige oliën, vlees en vis. Behalve deze twee zijn alle andere beschreven vormen waarschijnlijk pathologische of regenererende vormen van reukepitheel.
2. De tussenschotten van de membrana limitans olfactoria omgeven grotendeels de steuncellen, de reukcellen en uitvoergangen van de klieren van Bowman.
3. Van de vesicula olfactoria, zijnde het perifeer uiteinde der reukcel, ontspruiten $\pm$ 36—40 cilia olfactoria. Deze hebben dezelfde bouw als de respiratorische cilia, maar de axiale fibrillenbundel eindigt niet in het basaallichaam, doch gaat er door heen en door de vesicula olfactoria naar het intra-epitheliale gedeelte van de dendriet der reukcel. De basale dwarsplaten zijn onderling verbonden door donkere lijnvormige structuren.
4. Bij actief en volledig ontwikkeld reukepitheel vindt men veel secretoire pigmentgranula in de steuncellen en de kliercellen van Bowman. Het secreet van de klieren van Bowman zou van belang voor de reuk kunnen zijn. De kliercellen van Bowman worden in verschillende secretiestadia gevonden: het depôt-, het praesecretoire-, het secretoire- en het ruststadium.
5. Op de oppervlakte worden tubulaire protoplasma-uitsteeksels van de steuncellen

gevonden; deze uitsteeksels steunen de vesiculae olfactoriae met hun cilia. Analoge kleinere tubulaire uitsteeksels worden op de kliercellen en in de kliergangen gevonden. Ze hebben mogelijk een functie in verband met de secretie. Zij zijn niet identiek met de microvilli. Voorts worden bij pathologisch en regenererend reukepitheel z.g. micro-cilia gevonden, die kunnen ontstaan uit de tubulaire protoplasma-uitlopers.

6. Er wordt verondersteld, dat uit de kliercellen van Bowman de basale of vervangcellen ontstaan, die de aanstaande reuk- en steuncellen zijn. Bij herhaalde regeneratie na herhaalde heftige infectie zal door de mogelijke afwezigheid of afname van mitoses in de kliercellen van Bowman het depôt van deze cellen uitgeput raken. Dientengevolge kan de regio olfactoria verkleind worden en de reukzin afnemen.
7. De kunstmatig veroorzaakte regeneratie van de oppervlakkige structuren, o.a. de vesiculae olfactoriae en de tubulaire uitsteeksels der steuncellen, geschiedt op analoge wijze als de embryologische ontwikkeling. Er ontstaat een syncytium vlak onder de oppervlakte met centrioolachtige structuren. Ook groeit eerst het centrale uitsteeksel van de reukcel uit.
8. Er wordt bij de regeneratie van het reukepitheel der kat een indeuking van de reukkernen gevonden, mogelijk veroorzaakt door een uitgroeien van de steuncellen naar perifeer en mogelijke migratie van de reukcellen van de oppervlakkige naar de middelste lagen.
9. Bij regeneratie van het reukepitheeloppervlak van de mens na een oppervlakkige infectieuze beschadiging schijnt er een migratie te bestaan van de reukcelkernen naar de oppervlakkige laag om mogelijk met behulp van centrioolachtige vormsels de groei van de uitstekende structuren te induceren.

INTRODUCTION AND HISTORY

The ear, the eye, and the organ of smell are of the utmost importance to man and animal both for living and procreation. Birds receive most information through the eye and the ear. For mammals information obtained through the sense of smell is of great consequence. The olfactory sense in man is often referred to as being degenerated. This is certainly not true, the human olfactory organ is surprisingly sensitive as it can perceive, analyse and classify odours with a speed and accuracy which no laboratory instrument has been able to compete with up till now. The social and economic meaning of smell must not be under-estimated either. The gigantic money-investments in the perfume industry prove that odoriferousness is an important factor in human relations. Moreover the tasting of food is essentially a function of the organ of smell, the function of the tongue being restricted to distinguishing between the qualities: acidity, sweetness saltness and bitterness. Thus in total anosmia all wines taste like diluted vinegar. While we eat or drink the scent of the food reaches our organ of smell via the nasopharynx. Therefore the flavour of a dish and the flower of wine can only be fully enjoyed because of the existence of this "backdoor".

Animals are usually divided into macrosmats and microsmats, which division is based upon the relative size of the olfactory system in the brain. A study of the extension of the olfactory epithelium in the nose is also of great inportance to decide whether an animal belongs to the very sensitive or the moderately sensitive ones. Such studies are scarce. According to ADRIAN (1956) the surface of the olfactory epithelium in the small badger dog is about 20 cm^2, whereas a more accurate measurement by A. MULLER (1955) establishes this surface in the same dog as 74.84 cm^2. In man, the olfactory epithelium surface measures, according to EGGSTON and WOLFF, only 2.5—5 cm^2, so that it has about the same size as the sensitive area of the retina. This enormous difference is perhaps a better indication of the greater importance of this organ in dogs than in man.

Interest in the anatomy and histology of the olfactory organ dates already from ancient times. HIPPOCRATES ($\pm$ 400 B.C.), probably aware of the fact that the fila olfactoria pass through the lamina cribrosa into the nose, was of the opinion that the brains extended into the nose. He also knew that smelling was only possible if the olfactory cleft contained air. Somewhat later on RUFUS EPHESIUS (311 B.C.) mentioned the olfactory nerve. Not untill the middle of the nineteenth century was a first histological description of the olfactory epithelium given by ECKHART and ECKER (1855 and 1856). Both described the columnar structure of the epithelial cells, in which

ECKHART already distinguished two different types. ECKER assumed the cells with descending fibres to be replacement cells, but SCHULTZE (1862) considered them to be the nervous elements.

According to ECKER the epithelial cells branch out proximally to the connective tissue and meet ascending nervous elements with which they seem to make contact. SCHULTZE (1856 and 1862), studying the olfactory tissues of mammals and amphibians, saw filiform cells with varicose swellings in the central projections, which swellings were similar to those seen in the olfactory nerve; he called these cells olfactory cells. The central projections and the olfactory nervous tissue intertwined and formed a network. He supposed that there must be connections, but he was unable to demonstrate them. Staining methods applied later on would prove his speculations correct.

EXNER (1871 and 1877) and SCHULTZE differed in opinions. EXNER found many intermediate cells but he did not see that either of the cell types were directly connected with the olfactory nerves. It appeared to him that all cells were connected with a subepithelial network with which the nerves also came in contact. From this he postulated that both types of cells had perceptive functions. His theory was not generally accepted, and with the advent of the new staining methods he was forced to give up his point of view.

VON BRUNN (1875) described with great precision that the membrana limitans olfactoria lay on top of the supporting cells. Through this membrane penetrated the peripheral projections of the olfactory cells, while underneath it there was a network of partitions, originating from the membrana olfactoria.

EHRLICH (1886), working with methylene blue, showed that the central portion of the olfactory cell merged gradually into a varicose-shaped nerve fibre. CISOFF had made the same observation as early as 1874. These observations were subsequently confirmed by GRASSI and CASTRONOVO (1889) who used GOLGI's staining methods.

SCHULTZE had already described the knob-shaped swelling of the peripheral projection of the olfactory cell, the surface of which swelling he found to be covered with 6—10 very thin hairs. Many authors including SCHULTZE, believed these structures to be artefacts. VAN DER STRICHT (1909) gave this peripheral knob-shaped element the name of "Vesicula olfactoria". The structure of these vesicles has been investigated ever since, but because of their small size they are not easy to examine with the ordinary light microscope. KALLIUS (1905), KOLMER (1927), LE GROS CLARK and WARWICK (1946) found them to be permanent structures.

In adult man the olfactory epithelium is located at the concha superior and at the uppermost portion of the septum; in children also at the portion of the septum opposite to the concha media. In other mammals a large part of the upper concha and of the ethmoid turbinates is also covered with olfactory epithelium thus being a labyrinth of cavities and passages.

The electron microscope was introduced relatively late in the study of the olfactory organ. The first publication was by ENGSTRÖM in 1952, who had already published a similar study on the cochlea. Later on his pupil, BLOOM, published several articles

(1954), while recently a study by GASSER appeared (1956). ENGSTRÖM's publications appeared at the time that we had already started our investigations.

All investigations by means of high power magnifications have up to now only been concerned with the normal olfactory epithelium. Therefore we were of opinion that a study of pathological processes was highly desirable. We soon experienced, however, in studying the literature on pathological olfactory epithelium that even the textbooks of special pathology and those of general pathology, did not contain anything of importance. In accidentally found pathological olfactory epithelium we often saw pictures which resembled regeneration. This induced us to study the problem of the regeneration of damaged olfactory epithelium. Probably this is a more important problem in the pathology of the organ of smell than has generally been admitted up to now. So the very frequent nose infections and allergic reactions are supposed to cause anosmia by a temporary obstruction of the olfactory cleft. It is likely, however, that in these cases anosmia is also due to damage of the very sensitive olfactory epithelium. In addition permanent damage may be the result of misuse of nose drops. Anatomical studies have demonstrated that already early in life a certain damage of the organ of smell is present. For these different reasons it is not impossible that a certain capacity to regenerate protects this organ against early total destruction.

Because electron microscopical studies were scarce and incomplete, it was necessary to start our investigation by the study of normal olfactory epithelium. In order to study regeneration, the olfactory epithelium of cats was damaged and after some days or weeks the epithelium was examined. It also proved necessary to study embryology, because the same pattern seemed to be followed both in regeneration and in development.

PART I

ELECTRON MICROSCOPY OF NORMAL OLFACTORY EPITHELIUM

CHAPTER I

LIGHT MICROSCOPY OF THE OLFACTORY EPITHELIUM

The olfactory epithelium in men and animals is composed of three types of cells: supporting cells, olfactory cells and basal cells. In contrast to the respiratory mucous membrane covering the rest of the nose there is no membrana propria, and therefore the epithelium merges directly into connective tissue with blood vessels, nerves and Bowman glands. There is, however, a superficial area devoid of nuclei.

§ 1. THE OLFACTORY CELLS. These are bipolar cells which, morphologically, are ganglion cells, analogous to those of the spinal posterior root-ganglia. They stretch throughout the entire thickness of the olfactory epithelium and lie embedded between the supporting cells. The latter, according to LE GROS CLARK, are pentagonal or hexagonal on cross section, the nerve cells forming part of a circle around each supporting cell. The olfactory cells have a round nucleus lying either in their centre or in their lower part. The olfactory cell nuclei lie at varying levels: so the uppermost nuclei are found among the supporting cell nuclei and the lowermost among the basal cell nuclei. The lengths of the peripheral processes of the olfactory cells thus vary from 20—90 μ, their diameters from 0.6—1.5 μ. According to LE GROS CLARK and WARWICK (1946, 1950) the neurofibrils in the olfactory cells are tubular on cross-section. The central processes, which are smaller in diameter, form bundles under the olfactory epithelium and subsequently, surrounded by a sheath of Schwann cells, pass through the lamina cribrosa as the fila olfactoria and reach the bulbus olfactorius. Within the bulbus they are connected with the so-called glomeruli, resembling fibrous cells, and with the mitral cells, which are connected with the cerebrum. The peripheral end of the olfactory cell is a receptor organ: the olfactory vesicle probably is a type of modified dendrite. Each nerve fibre bundle forms a single system with its receptor and there are probably no anastomoses among the fibres.

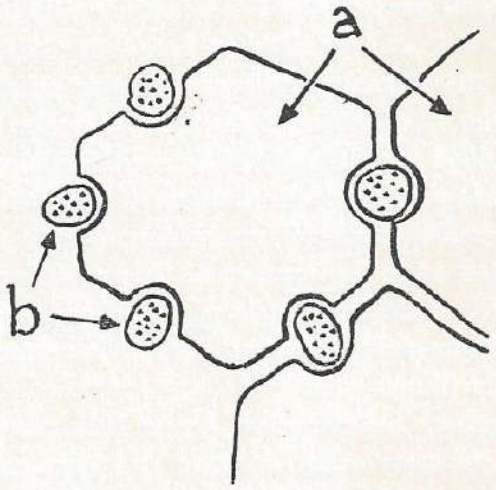
a. *Quantitative investigation.* According to ADAM MÜLLER (1955) there are in the badger dog 124,289,450 olfactory cells. ALLISON and WARWICK (1949) found in each half of the nose of a four month old rabbit 50 million receptors. Posteriorly the density decreases. In one bulbus olfactorius they found approximately 1900 glomeruli, 45,000 mitral cells and 130,000 tufted cells, and so one glomerulus receives impulses from about 26,000 receptors and passes them on to 24 mitral cells and 68 tufted cells.

b. *The vesicula olfactoria.* In the true sense of the word these are not vesicles. In the light microscope they show as elevations of varying sizes of the epithelium surface,

interiorly vacuolated. Their surface is covered with thin hair-like elements, named cilia olfactoria (fig. 1). These are 1—2 μ in length and on cross-section 0.1 μ thick. According to VON BRUNN (1880) in man there are between 5—6, in the pig 5—8 of these cilia, and according to LE GROS CLARK and WARWICK (1946) in the rabbit 10—14. Owing to the extremely small size of the vesicles there is little agreement between the descriptions of the cilia given by various authors. They have been described both as one thick flagellum emanating from the vesicle, and by LE GROS CLARK as a bundle of hairs at the end provided with small knobs.

§ 2. THE SUPPORTING CELLS are large and protracted and are found throughout the entire thickness of the epithelium. Peripherally they form a rather thick layer. According to EXNER (1871) the uppermost layer bears cilia similar to those of the sensory cells; ECKHARDT (1855) and COLOSANTI (1875) came to the same conclusion (fig. 1). HOLMFELD (1883) recognizes two types of supporting cells, one with and the other without cilia. VAN DER STRICHT (1909) saw cilia upon the supporting cells in the trout embryo. The cells have a centrally situated oval nucleus lying in different horizontal planes, usually located in the upper third of the cell. The central process is conical and thinner than the peripheral process, and according to FINDLAY (1894), EXNER (1871) and MARTIN (1873), it branches into a subepithelial network.

The most conceivable description seems to be that of VON BRUNN (1880). He sees the supporting cells as cylinders excavated at their sides, with the olfactory cells lying in the excavations. The supporting cells may be indented in such a way as to come into close contact with the projections of the adjacent supporting cells. So the supporting cell is the core of the olfactory cell bundle.



Drawing 1. Schematic representation of a cross-sectioned supporting cell (*a*). The cross-section of this cell is hexagonal. The supporting cell is the core of the olfactory cell (*b*) bundle. The olfactory cell indents the supporting cell at its sides.

Around the nucleus of the supporting cell a Golgi network is visible. ALLISON and WARWICK (1947) saw laterally projecting tonofibrils, arising from the supporting cell,

together with basal bodies and secretion products, as did LAMS (1940) and PLANEL (1951). Moreover, the presence of large numbers of granules arranged in rows gives the supporting cells a striped appearance (fig. 2).

§ 3. THE PIGMENT. Besides being thicker than the rest of the mucous membrane of the nose, the olfactory epithelium can often be distinguished macroscopically by its colour. In sheep the epithelium is yellowish-brown, tinged with black, it is yellow in the dog, brown in the adult cat, and in man it sometimes is yellowish, but usually it does not differ appreciably in colour from the respiratory epithelium.

SCHULTZE (1856) saw pigment in cells of the olfactory epithelium in man and some adult animals, whereas in young animals pigment is smaller in quantity than in older ones of the same species. The pigment is found principally in the supporting cells. In man and in cavia it is located in the peripheral and the middle part of the cellbody, while in the dog, sheep, cat and horse the greatest concentration is found in the deeper layers near the border of epithelium and connective tissue (fig. 2 in cat and fig. 15 in men).

Lying closer to each other than in the supporting cells, the pigment granules can also be found in the Bowman glands, where they have a secretory function. The importance of the pigment with regard to the functioning of the olfactory sense was evidenced by the electro-physiological investigation of ADRIAN (1956), who demonstrated that areas with great amounts of pigment are more sensitive and not so easily exhausted as those containing smaller quantities of it. It is well known that the sense of smell of albinoes is poor or even absent, while negroes, who lose their pigment, also lose their sense of smell. So the pigment appears to be essential to the olfactory function. The parallel arrangement of the pigment granula as seen in fig. 2 was attributed by FINDLAY (1894) to fine olfactory fibrils enveloping the large supporting cells.

§ 4. MEMBRANA LIMITANS OLFACTORIA OF VON BRUNN (CUTICULAR MEMBRANE). According to EGGSTON and WOLFF (1947), the supporting cells are covered at their peripheral end by a flat cuticular membrane apparently composed of small prickles and thorns with a semi-liquid substance in between. In this membrana limitans there are small openings through which the sensory elements emerge. According to VON BRUNN the lower side of the membrane is composed of projecting partitions which penetrate among the supporting and olfactory cells and in this way form a sort of network. In the membrana limitans short canals exist, through which canals the peripheral ends of the olfactory cells pass perpendicularly. These canals have diameters equal to or slightly larger than those of the peripheral prolongations of the olfactory cells. There have been found knob-shaped and sometimes perforated swellings at the surface of the membrane, the peripheral neurons penetrating through the perforations. VON BRUNN (1875) found this structure only after a 2—10 hours fixation with 0.5—0.25 % solution of osmic acid. After a 0.005 % chromic acid solution fixation the membrane was no longer visible. He saw only a fine film stretched over the epithelium (fig. 2), and assumed that the membrane had been either dissolved or not fixated.

KRAUSE described the membrane as a layer of rudimentary cilia, analogous to that

of the cells of the intestinal epithelium. VON BRUNN (1880) recognized the minute hairs on the epithelium as an olfactory membrane. He described yet another cuticular membrane under the projecting hairs together with the intercellular system of partitions. KALLIUS (1905) saw only this intercellular connecting pattern and the hairs on the supporting cells. VAN DER STRICHT (1909) described a transparent or reticular membrane resting on the supporting cells, arising from a network of partitions. EXNER (1877), KALLIUS (1905) and SUCHANNEK (1892/3) argued that the membrane was merely an artifact, resulting from agglutination of the olfactory cilia by a serous fluid.

Some investigators are of the opinion that the cuticular membrane consists of protuberances similar to those of the intestinal epithelium; others that it consists of fine cilia, and still others that it consists a small slim pegs.

§ 5. HAIR-SHAPED SURFACE STRUCTURES. SCHULTZE (1862) found two types of hairs originating from the surface, which hairs were twelve times as long as ordinary cilia ($\pm$ 135 μ.). The first type consisted of long thin hairs, slightly thicker at their bases, some making slight swaying motions, whereas the second type consisted of stiff and motionless hairs. There were areas provided with movable cilia only, others with only unmovable cilia, and some with both types. Immersed in water they degenerated to fine grains, whereas cilia in a non-sensory epithelium kept moving for hours. In the frog he found three types; long immobile hairs, hairs of medium length moving slightly during a short period, and clearly moving hairs. This was later affirmed, in studying in vivo preparations from frogs, by HOPKINS (1926). He saw long motionless cilia, distally thin, basally thick, 75—150 μ long, and mobile short ones with a length of 20—50 μ. The diameters of the two types differed. The movements of these cilia were not similar to the movements of ordinary cilia. They moved slowly, irregularly, not limited to one plane, and could not make beating movements. The individual hair curled from its top down to its base, after which it again assumed the vertical position. Another type of movement was a flexion of the proximal part of the hair towards the surface of the epithelium, the curve subsequently travelling to the top of the hair. At the end of this movement the hair lay parallel to the surface of the epithelium. The two sorts of hairs either were present in the same quantities, or one type predominated. Chloroform, ether, alcohol, and probably several other substances reduced the length of the hairs to 35 μ by dissolving their lipoid content. The time in which this was accomplished differed. One could occasionally see lysis of the supporting cells rather than of the olfactory elements. This made HOPKINS wonder whether the membrana limitans olfactoria of VON BRUNN was thinner than the lipoidal stratum of the olfactory hairs. The disposition of the hairs to be easily dissolved probably explains why they are so extremely short, both when seen in light- and electron microscopy. Preparations of human olfactory mucosa obtained at operations are no doubt superficially damaged by cytolysis as a consequence of solution of the olfactory hairs and cuticular membrane by inhalation anesthesia and/or fixation methods. The mucous layer is 10—60 μ thick, averaging 25 μ, therefore, according to HOPKINS, the olfactory hairs cannot but extend well beyond it. If water enters the nasal cavity there is an increased production of mucus

to compensate the change of osmotic pressure, and the hairs will be solved. From both causes smell can be lost.

§ 6. THE BASAL OR REPLACEMENT CELLS. These have small nuclei. They are closely attached to the fibrils of the supporting cells and the subepithelial connective tissue. FINDLAY (1894) described two types of projections: a proximal process provided with subepithelial fibrils, and a distal projection that does not reach the surface and ends conically in branches. He compared them to multipolar nerve cells. They were aptly called replacement cells since they seemed capable of changing into either supporting or olfactory cells. PLANEL (1951) was of the opinion that they were replacement cells for the cells of the Bowman glands. Anyhow, many investigators saw mitoses in the basal cells. Sometimes the basal cells are absent, in which case the lower ends of both the supporting and sensory cells lie next to the subepithelial tissue.

§ 7. THE BOWMAN GLANDS which lie in the subepithelial tissue, are branching tubulo-alveolar glands with yellow pigmented secretory granules. They probably produce a mucus-like substance. Sometimes the glands slightly extend into the epithelium (see arrow in fig. 2). PLANEL saw this in a non-secretory portion of a rabbit's gland which lay intra-epithelially. The discovery of intra-epithelial blood vessels by BAKKER (1939) and PLANEL (1951) can also be explained by the absence of a membrana propria in the epithelium.

FINDLAY saw large spheroidal cells with many granules in the alveolar portions: further on, the cells became more polygonal in shape, and still further, near the mouth of the gland, they were cubical and small; the glands themselves discharged on the surface. SLOTWINSKY (1934) saw no mucous granula in the upper part of the gland tubuli, in the lower part he saw many.

PLANEL distinguished 4 types of Bowman glands occurring in different species of mammals.

1. In abundant subepithelial tissue he found glands with transversely broadened cells and ducts with a wide lumen. These he found in the rabbit's septum.
2. Wherever there was little subepithelial tissue, he regularly found tubes with a very small lumen and prismatic cells of equal thickness; these he usually found in the septum of the guinea pig.
3. In the case of abundant olfactory bundles and little subepithelial tissue he found flattened tubes with cells that overlapped each other. These were found in the posterior part of the nasal cavity of the guinea pig and the rat.
4. Finally he described polymorphous glands in the superficial loose tissue, with cells that are narrow at the base and wide towards their surface. In the deeper layers where there were many nervous elements, the structure was that of a tube lined with one layer of cells overlapping each other. These structures were found in the rat. PLANEL does not describe human Bowman glands.

Occasionally PLANEL saw signs of degeneration and pycnotic nuclei within the Bowman gland cells. Finally they completely disappeared; their place was taken by neighbouring gland cells. According to GEREBTZOFF and SHKAPENKO (1952) all animals

Figure 1. Olfactory epithelium of cat. I. Magnified 1100—1200 × (nervous tissue staining method of Bodian). Light microscopic photograph. Within the nuclei-free section of the olfactory epithelium the peripheral processes of the olfactory cells can be seen, which processes end in a hemispherical vesicle (arrow 1). 6—7 hairy projections seem to protrude from the surface of this vesicle. There are also common cilia visible on the supporting cells. (arrow 2).

Figure 2. Olfactory epithelium of cat I. Magnified 500—600 ×. Bodian stain. Light microscopic photograph. In the area devoid of nuclei the peripheral endings of the olfactory cells can be seen. More basally the pigment granules are lying in rows (arrow 1). Under these the Bowman glands with granules and a Bowman gland entering the epithelium are visible (arrow 2).

Figure 3. Electron microphotograph of a perpendicular section of the olfactory epithelium of cat II. Type I. Magnified 2000 ×. In comparison with figure 1 we can clearly see that the vesicles (arrow 1) contain no vesicle structure and lie mostly above the surface of the supporting cells. The vesicle on the extreme left has slightly sunk into the surface and is connected with a long peripheral process of a neuron whose nucleus lies in the deeper half of the olfactory epithelium. The membrana limitans is visible as a fibrous network. The supporting cell nuclei lie more peripherally (arrow 2) in a linear arrangement, a few being situated in the middle and in the basal part of the epithelium. The more electron-dense neuron nuclei (arrow 3) lie spread between the middle and the basal part of the supporting cells. The linear arrangement of the granula can be seen especially in the central process of the supporting cells. The central process of the olfactory cell is only rarely found in this perpendicular section. There are few basal cells present.

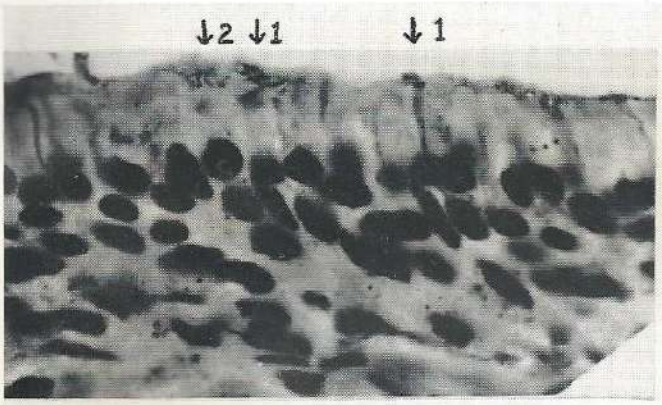


Fig. 1

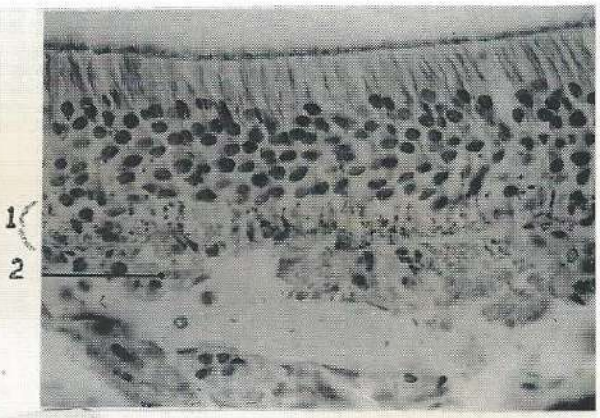


Fig. 2

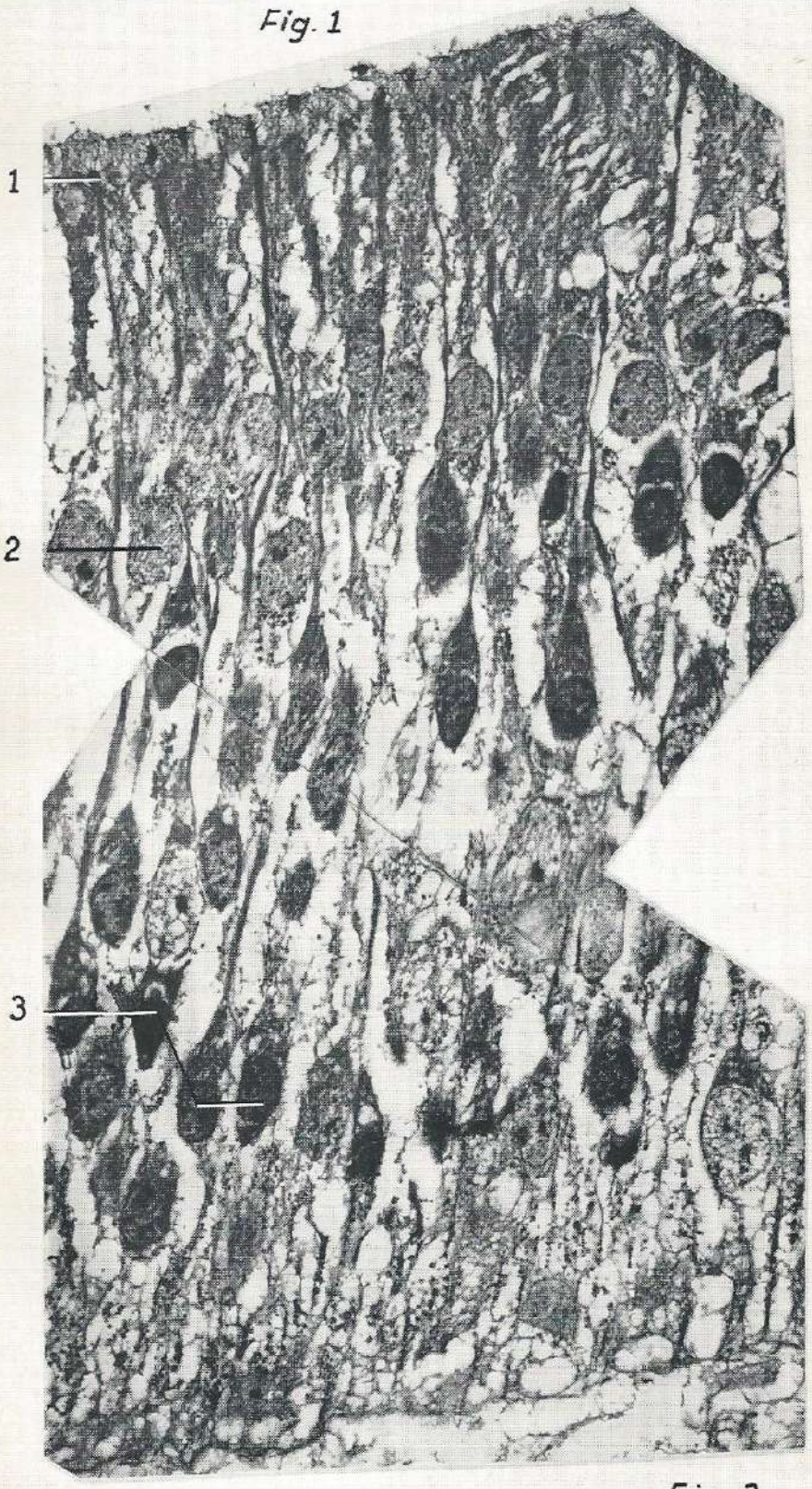


Fig. 3

with a pulmonary respiratory system have Bowman glands and therefore fishes were supposed not to have them. SCHULTZE (1856), however, described mucous glands in fish.

According to LAMS (1947), SLOTWINSKY (1932) and PLANEL (1951), the secretion produced by the Bowman glands is mucous. This has been demonstrated by staining methods. PLANEL failed to see the demilunes of GIANUZZI, neither did he see a membrana propria or muscular or collagenous layers around the acini. The glands lay in the subepithelial tissue. In the rat and the mouse, however, PLANEL could find no excretory canals, in contrast to the guinea pig and the rabbit. Although at the level of the basal layer these canals were still present, at a higher level of the epithelium the secretion could be found only interstitially. KALLIUS (1905) reported that the Bowman glands empty into ciliated crypts; others, however, have doubted this. SLOTWINSKY (1935) described cells at the bottom of the gland, not so large as the others and lacking both granula and mitochondria. He supposed them to be either exhausted, or undifferentiated cells, which will eventually develop into mature olfactory epithelium cells.

CHAPTER II

MATERIAL AND METHODS

The olfactory epithelium examined was limited to that of man and the cat, chiefly the cat. In the material removed during operations, mainly performed on patients suffering from cancer, it was possible to find olfactory epithelium. Unfortunately, as is the case with the cochlea and other sensory epithelia, late post-mortem material can not be used, because of autolysis developing very rapidly. Olfactory epithelium from chronically sick patients is pathologically changed and thus mostly unsuitable. The mucous membrane of the upper concha and the ethmoid cells mostly appeared to contain only respiratory epithelium. In the mucous membrane of the septum, on the other hand, olfactory nerve elements were found. Consequently, of the human olfactory mucous membrane only the septal part will be dealt with. The absence of nervous epithelium in other places in the superior nose cavities of our patients may possibly be attributed to degenerations resulting from previous infections. In the cat were examined the olfactory mucous membrane of the maxillo-turbinates as well as that of the ethmoid-turbinates and of the septum. Rapid fixation of the tissues is the first requirement for electron microscopy, even more so than for light microscopy. In material obtained during operation, the inhaled anaesthetic, the locally applied anaesthetic or haemostatic agent, as well as the use of diathermy, can cause damage and make the epithelium unsuitable.

The cats were anaesthetized by an intraperitoneal injection of evipan. Following this the ossa nasalia and a portion of the alae nasi were removed and the regio olfactoria posterior in the nasal cavity and the ethmoid were laid bare. Especially with regard to the superficial structures the best results were obtained when the olfactory epithelium was fixed in vivo with veronal acetate buffered 1 % osmiumtetroxyde solution at a pH of 7.2. Fixation was effectuated by dripping the fixative on the epithelium as well as by submucous injections. A few cats were transfused in the aorta with saline to free the tissue from erythrocytes; a few others (Cats XIII, XIV, XV) were not transfused and were only locally fixed with an isotonic 1 % solution of osmic acid for 1—1¼ hours.

In the beginning the preparations of cats II and III were embedded in a mixture of paraffin and beeswax. Later on they were embedded in a mixture of butylmethacryolate and methylmethacryolate polymerised with 1 % dichlorobenzoylperoxide.

In a few cats (cats IV, X, XII) the anterior, the posterior, the superior, and inferior parts of the olfactory area of the septum and also of the conchae were separately in-

vestigated. This was done because we found different forms of the olfactory epithelium in our preparations.

Sections were cut to a thickness of 0.2 μ and with the Philips ultramicrotome to a thickness of $\pm$ 200 Å. Next they were lifted by means of silver object carriers and examined with a Philips electron microscope type EM 100. Since one picture represents too small a part of the cross-section to give a reasonable view, several photographs were made and mounted together. Parts of the olfactory epithelia of fifteen cats and three humans have been investigated. The epithelia of cats I, II, III, IV, X, XI, XII had not pathologically changed; accordingly neither pus nor mucus was found in the nasal cavities of these animals.

The olfactory epithelium of Homo I was supposed to be normal, because there was seen no pus or mucus in the nose cavities. The surface of the epithelium, however, was partly dissolved or destroyed, possibly by the inhalation anaesthesia. In all other cats and humans the olfactory epithelia were abnormal.

CHAPTER III

THE OLFACTORY EPITHELIUM AS SEEN WITH THE ELECTRON MICROSCOPE

§ 1. THE OLFACTORY EPITHELIUM. The olfactory epithelium of cat I has been prepared for light-microscopical investigation, the epithelium of cats II, III etc. for electron-microscopical examination.

In the aforementioned light microscope picture of cat number one's olfactory epithelium (fig. 1) the vesicles protrude mostly above the surface of the cells, though as can be seen in the electron-microphotograph of the olfactory epithelium of cat II (fig. 3, Arrow 1) a vesicle may occasionally lie in a small surface depression; mostly the vesicles protrude, as is also seen in the epithelia of cats IV, X, XII. A vesicle is not actually a blister, for there is no cavity to be seen in it. The olfactory vesicles lie in the middle of the so called "cuticula", clearly visible as a fibrous network in fig. 3. — We shall describe and discuss the cuticula in § 6 —. The so called modified dendrite can easily be followed to the more osmiophilic — more electron-dense — neuron nucleus, 4—4,5 μ in diameter. Above the nucleus in the peripheral cell process a Golgi network is visible. A perpendicular cut, as depicted in fig. 3, just includes the nucleus and the peripheral projections of the olfactory cells, but a central projection is rarely seen. Superficially a layer of oval supporting cell nuclei, 5—6 μ in diameter, is visible and under this lies a layer of neuron nuclei, 4—4,5 μ in diameter, while deeper both supporting cell and neuron nuclei are found among the few basal cell nuclei. Some pigment granules are seen, they are mainly found basally. The basal cells are almost entirely absent here, and the lower parts of both the supporting and the olfactory cells mostly lie next to the subepithelial tissue.

In the olfactory epithelium of cats III, XI and also of cat XIII, in contrast to the above described epithelium, most vesicles are found in the valleys formed by the walls of the supporting cells (figs. 4, 5, 36 and 37). However at places a vesicle may partly protrude beyond the surface, whereas evidently their cilia always do. A characteristic of this type is the absence of an uppermost layer of supporting cell nuclei, contrary to the findings of other authors and to what has been described above. Instead there is a superficial layer of osmiophilic electron-dense neuron nuclei, 4—5 μ in diameter. In figure 5 two vesicles can be seen in one depression.

The superficial portion, devoid of nuclei, is conspicuous by its syncytial structure and its electron-dense filiform structures lying in the lengthening of the electron-dense cell nuclei. These hills formed by the supporting cells contain very few mitochondria:

peripherally there are none at all. Under the layer with neuron nuclei there are large numbers of supporting cell nuclei, each surrounded by a Golgi network. This network forms such a firm skeleton as to indent the olfactory cell nuclei (fig. 4, Arrow 4). Basal cells or replacement cells are already seen halfway the thickness of the epithelium. Among them there are also found a few polymorphous, osmiophilic, more electron-dense nuclei. These elements and the other replacement cells will be discussed in the second part of this study. Finally, various kinds of granules are found to lie in the protoplasm of the basal portion of the supporting cells.

Some preparations of cat III which, accidentally, were cut oblique to the surface, provided us with another picture of the olfactory epithelium. Here a cuticular structure was found near the surface of the epithelium. We found partitions between the supporting and the olfactory cells which pass through three quarters or more of the epithelium thickness and completely or almost completely surround the olfactory neuron. These partitions can form a network around the supporting cells (fig. 6). This network merges into a large cuticula immediately under the surface where (fig. 7: arrow 1) this cuticula is pierced by the peripheral processes of the olfactory neurons. Within these processes, long filiform structures are found. It appears, however, that the partitions surround not only the intra-epithelial part of the peripheral process of the neuron, but its entire length with a great part of the neuronal perikaryon and occasionally a part of the central axon. The centre of figure 6 (see arrow 2) even shows a syncytium with three olfactory cell nuclei, the peripheral and central processes of these cells appearing to be intimately connected. The partitions themselves have projections into the supporting cells. Occasionally they enclose small parts of the supporting cells, which enclosed parts appear as holes in the partitions (fig. 6, arrow 4). These holes are filled with the protoplasm of the supporting cells with at places a round granular mitochondrium. This partition network and the membrana olfactoria both seem to consist of a dense, apparently amorphous, intermediate substance, within which can be seen darker granules (fig. 17).

In cat III we have also found olfactory epithelium without a cuticula; the localisation of the cuticula in the olfactory area could not be reconstructed afterwards. For this purpose the anterior, the posterior, the superior and the inferior of the olfactory area of the septum and also of the conchae have been separately investigated in a few cats (cats V, X, XII). No pus or mucus has been found in the nose cavities. The cuticula appears to be present only in the posterior part of the olfactory area of these cats. In this area, in the basal portion of the epithelium, we have found large quantities of pigment in the supporting cells as well as in the Bowman glands.

§ 2. THE OLFACTORY CELL BODY. The diameters of the olfactory cell nuclei range from 4—5 μ. The nucleus has a very dark appearance when osmic acid has been used as a fixative; it is sometimes filled with all forms of chromatin structures, far more densely packed than in the supporting cell nucleus, and it contains one or two nucleoli. In general the cells are bipolar, except in the olfactory epithelium, provided with a cuticula, where they sometimes seem to be multipolar, their axons joining those from other neurons (fig. 6 arrow 2).

Figure 4. Olfactory epithelium of cat III. Type II. Electron microphotograph. Magnified 1600 ×. The vesicles are found in the valleys (arrow 1) formed by the supporting cells that are covered with hairy projections. Noticeable is the absence of electron-dense structures in the most peripheral part of the supporting cells (arrow 2). Superficially in a syncytium the dark, electron-dense olfactory cell nuclei in whose lengthening filiform structures and vesiculae olfactoriae are visible (arrow 3). The olfactory cell-nuclei are oval or elongated. In the left-hand part of the picture olfactory cell-nuclei are indented by the supporting cells (arrow 4). The vertical arrow (1) points to two vesicles within one depression, arrow 5 to the cut duct of a Bowman gland. The inside of this duct is covered with the same type of fibrous projections as the surface of the supporting cells. The walls are formed by both supporting and basal cells. Notice basally the very large number of basal cells and the few cells among them with polymorphous, osmiophilic nuclei.

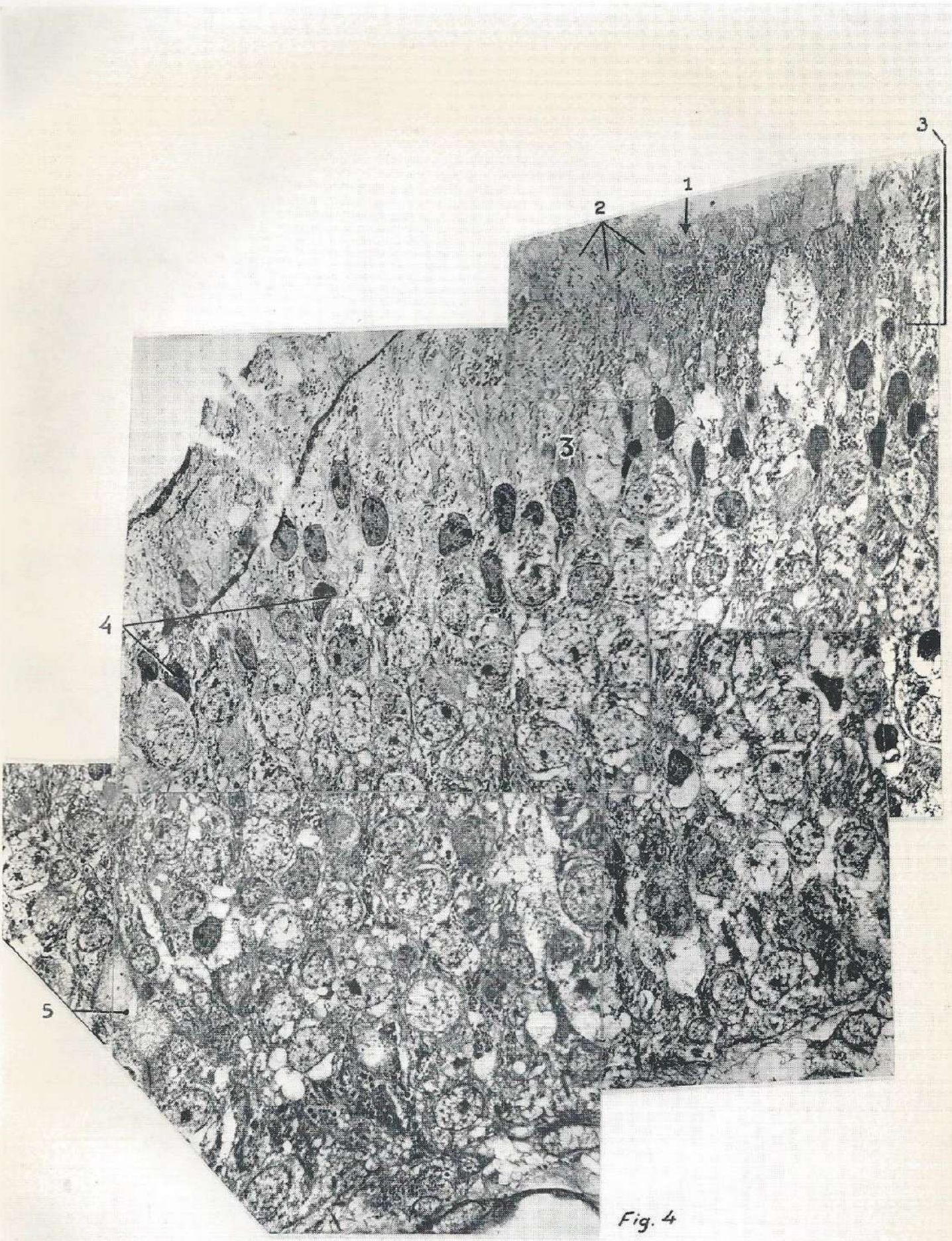


Fig. 4

In the olfactory epithelium described firstly (cat II) the olfactory cell nuclei lie under the first row of supporting cell nuclei (fig. 3) about in the second third part of the epithelium ($\pm$ 30 μ under the surface). In the epithelium described secondly (cat III) they lie closer to the surface (20—25 μ from the top) (fig. 4) and in the olfactory epithelium containing a cuticula they are found again immediately below the row of supporting cell nuclei. The lengths of both the peripheral dendrite and the central axon are variable. So the nuclei lie at different levels in the olfactory epithelium. Within the peripheral dendrite there are long filiform electron-dense structures (figs. 6, 7 and 8). In the terminal part of the dendrite, up to $\pm$ $\frac{1}{2}$ μ under the origin of the vesicula olfactoria, no mitochondrial or other electron-dense structures are present. The peripheral and central processes retain the protofibril structure, the protofibrils having a diameter of from 100—200 Å and lying from 200—300 Å apart. Close to the olfactory cell nucleus, the peripheral process of the cell broadens into the perikaryon with a Golgi network, while the protofibrils enclose the nucleus. Between and in the lengthening of the protofibrils various dark thread-like structures are present (fig. 8). Fibre-plexus of axons are occasionally seen in the lower layers of the olfactory epithelium (fig. 3) sometimes forming a syncytium (fig. 6 arrow 2). The peripheral dendrite ends in the vesicula olfactoria.

§ 3. THE VESICULA OLFACTORIA is the peripheral end of the dendrite. It exhibits differences in size, diameter and shape. Two shapes are generally seen, one oval, about 1.5—2 μ in diameter and 3 μ in length (fig. 11), and the other fingershaped, $\pm$ 4 μ in length and 1 μ wide (fig. 9). The cilia olfactoria have often lost part of their length and usually cannot be traced over more than 2—3 μ. They sprout from a round or elongated basal body resembling the diplosom of a vibration hair. The distances between the basal bodies ranges from 0.1—0.4 μ and in cross-sections of the vesicle they appear to be interconnected by electron-dense linear structures (figs. 10, 12 arrow 3 and fig. 14, arrow 2).

In a cross-section we see at least 8—9 cilia olfactoria or basal bodies. In a longitudinal section there are about 10 of them, in figure 9 even 19. Therefore at least 36—40 olfactory hairs may originate from each vesicle. The cilia sprout from the vesicle in a fan-shaped formation (fig. 9 and 11), only the uppermost cilia proceeding perpendicular to the surface. We see all the cilia rise upwards when they come from a vesicle lying deep in a valley (fig. 5); after a short distance they bend and run parallel to the surface. When the vesicle extends beyond the surface of the supporting cells, the cilia olfactoria do not proceed perpendicular to the surface, but almost immediately curve and run parallel to this surface (fig. 11), closely peripheral to the tops of the supporting cells. The cilia are supported by the supporting cell projections, with which they do not seem to have any kind of connection (fig. 9 and 17).

We find an inner structure of the cilia olfactoria similar to the common kino-cilia found in the whole fauna and flora: an axial fibrillary bundle surrounded by a thin sheath of granular structure. The bundle appears to consist of an outermost layer of nine paired fibrils with two fibrils in the centre. All of the fibrils are of the same diameter

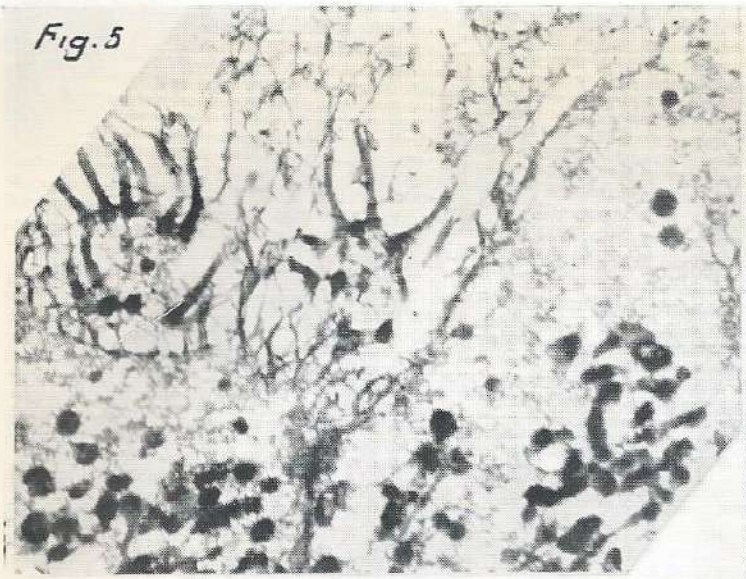
Figure 5. Olfactory epithelium of cat III. Type II. Magnified 10,000 ×. Two vesicles within one depression formed by the walls of a supporting cell covered with hairy projections. The connections between the diplosomes in this tangentially sectioned vesicle are visible only in a direction parallel to the surface.
All cilia olfactoria proceed straight upwards perpendicular to the surface. The absence of electron-dense structures in the peripheral part of the supporting cells, from which the protoplasmic projections originate, is striking.

Figure 6. Olfactory epithelium of cat III. Type III. Magnified 3000 ×, oblique section. The VON BRUNN partitions surround for the greater part the peripheral dendrites, which contain filiform structures (arrow 1), and they also surround the axons and the olfactory cell nuclei. On the surface club-shaped swellings are seen from which spring protoplasmic projections. These swellings are not vesicles; the latter are mostly absent because of damaging. The arrow (1) indicates a complex of partitions in the centre of which several small canals are left open in each of which lies a peripheral dendrite. The arrow (2) points to a syncytium with a group of olfactory neuron nuclei, in the centre of the picture. The supporting cell nuclei are round in this section (arrow 3), while the olfactory cell nuclei are protracted and oval (arrow 2). The partition projections into the supporting cells are especially perceptible near the surface (arrow 4). The supporting cell protoplasm contains various membranes of the endoplasmatic reticulum, between which membranes lie pigment granula (arrow 5).

Figure 7. Olfactory epithelium of cat III. Type III. Magnified 10,000 ×. Oblique section. The membrana limitans olfactoria is pierced by the peripheral dendrite (arrow 1) which is surrounded by a cell-membrane; the membrana is also connected with the peripheral portions of the supporting cells in which round mitochondria were visible and around which lies a clear cell-membrane (2). The surface is covered by projections arising from the membrane, sometimes stuck together. Only two vesicles can be seen (arrow 3). Within the peripheral dendrites filiform structures and protofibrils are visible.

Figure 8. Olfactory epithelium of cat III. Magnified 10,000 ×. The peripheral process of an olfactory neuron with protofibrils. The electron-dense filiform structures (arrow 1), in whose lengthening lie the protofibrils (arrow 2), are spread in osmiophilic protoplasm.

Fig. 5



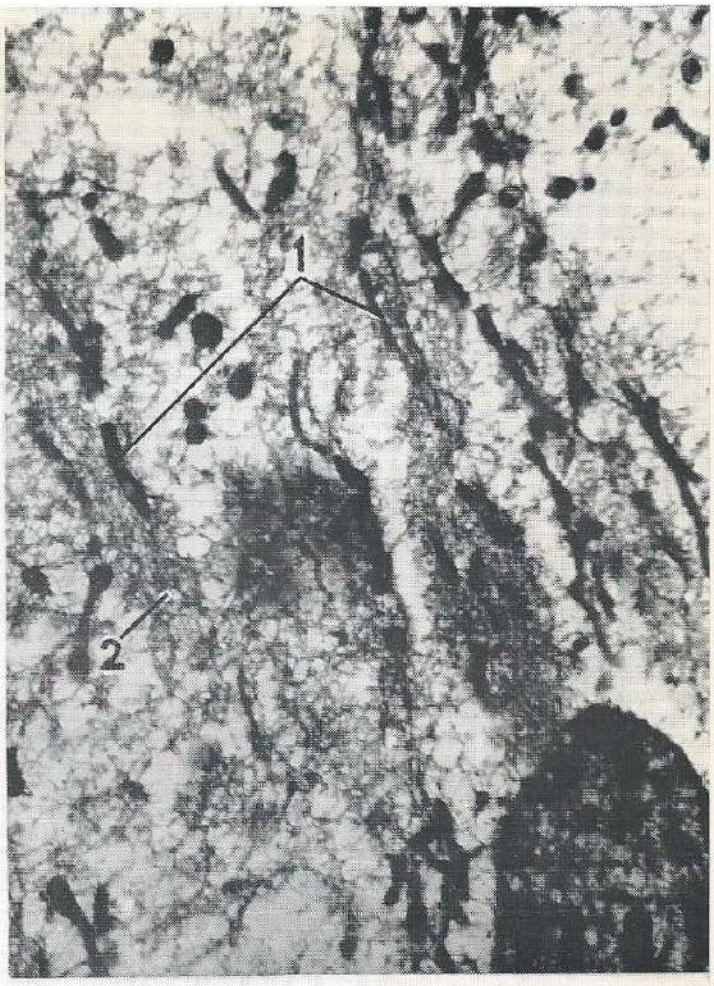


Fig. 8

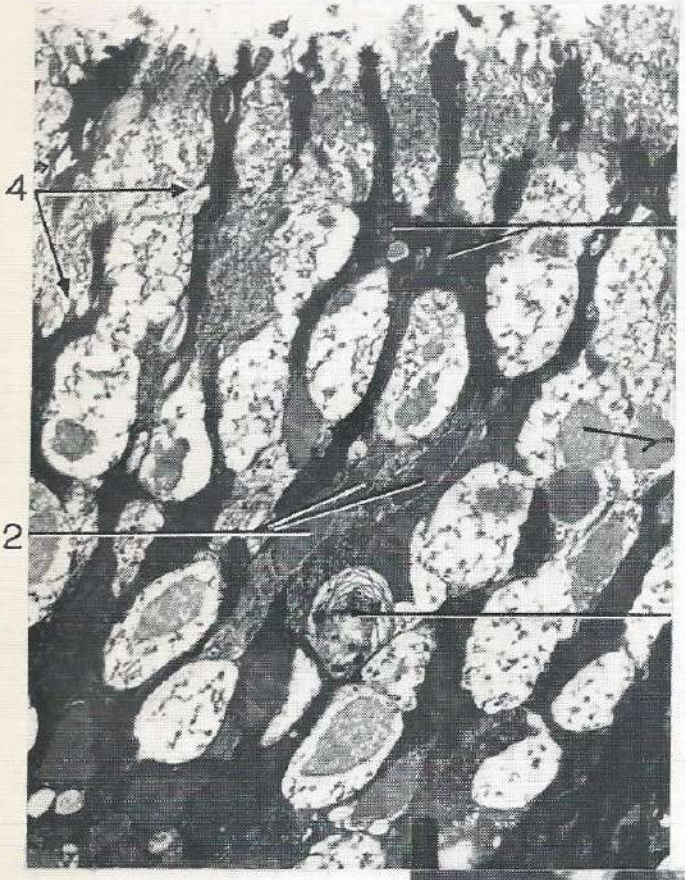


Fig. 6

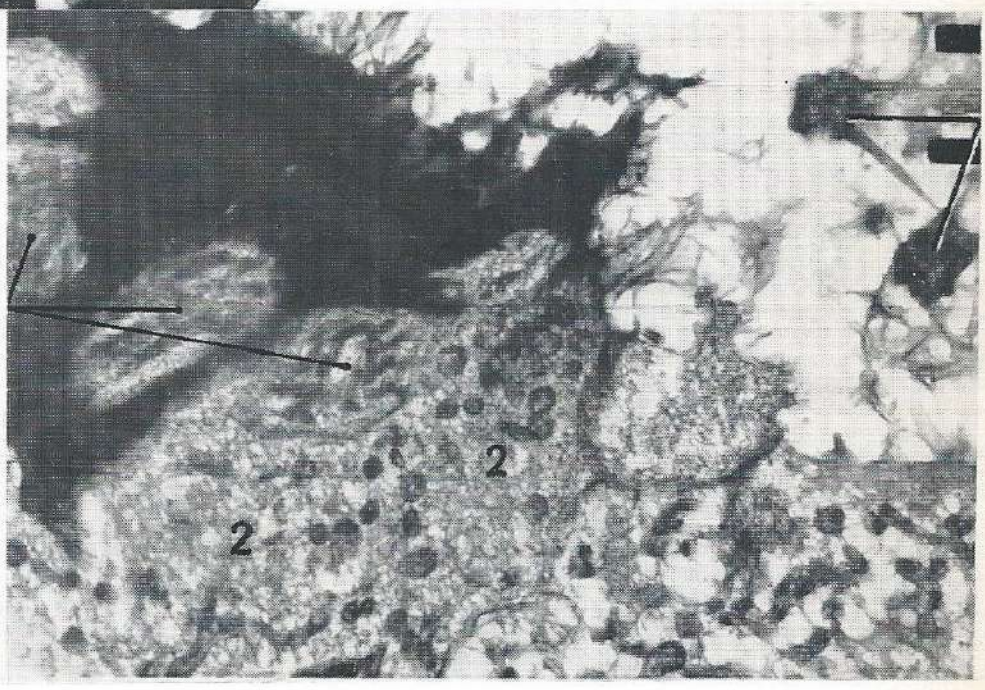
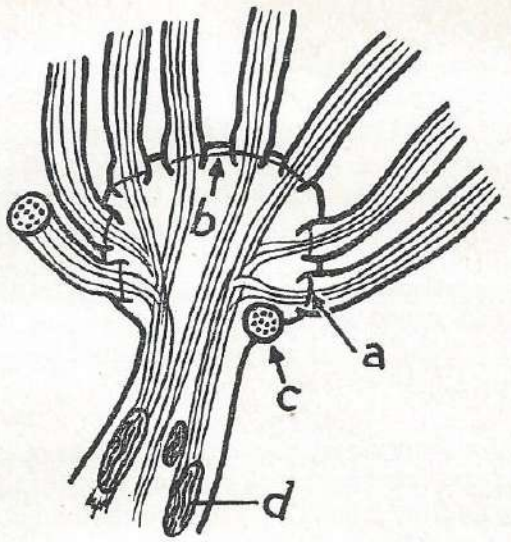


Fig. 7

± 150 Å. Moreover occasionally we saw, as shown in fig. 12 (arrow 1) and 14, a fibril bundle proceeding through the basal body into the vesicle. Passing through the root of the vesicle the fibril bundles reach the intra-epithelial part of the dendrite and continue between or within the filiform structures (fig. 11). A connection is made from the intra-epithelial part of the olfactory neuron to the olfactory hair via the vesicle and the basal body (fig. 13). Within the basal body at the lateral side of the vesicle the fibril bundle of the cilium makes an angle of ± 120°—145° (fig. 13) with its fibril bundle within the vesicle. There is a thin layer of cuticular protoplasm covering the vesicle, which layer merges into the thin sheath around the cilia olfactoria (fig. 12, arrow 2 and fig. 14, arrow 1).



Drawing 2. Diagramatic representation of an olfactory vesicle. From the filiform structures (*d*) of the peripheral part of the dendrite, several fibril bundles seem to emanate. First these fibril bundles travel together, then they continue as separate fibril bundles, until they reach the basal body, where an olfactory hair provided with an axial fibril bundle originates from the basal cross-plate (*a*). In the continuation of this cross-plate is found a structure interconnecting the basal bodies. These structures (*b*) lie only in planes perpendicular to the axis of the vesicle. *a*. cross-plate, *b*. interconnecting structure, *c*. cross-sectioned cilium, *d*. electron-dense filiform structures.

§ 4. THE SUPPORTING CELL. This cell stretches through the entire thickness of the olfactory epithelium. Its infranuclear part may vary in shape from conically narrow to ramifying in various processes. On cross-section the peripheral portion appears to be hexagonal (fig. 40). The peripheral dendrites squeeze themselves in between the corners of the hexagon (fig. 40 and drawing 1). The supporting cell nuclei are predominantly found in the upper third part of the thickness of the epithelium; in the epithelium described secondly (cat III), however, they lie in the middle third part. The supporting cell nucleus is less opaque than the olfactory cell nucleus and is provided with one or

Figure 9. Olfactory epithelium of cat III. Type II. Magnified 20,000 ×. Finger-shaped olfactory vesicle. The cilia olfactoria 1.5—2 μ long, diameter 0.3 μ, proceed from a basal body and rest upon the network of the supporting-cell projections. There are ± 19 basal bodies and/or cilia olfactoria present. An axial fibril bundle can be seen in both the uppermost and lowermost cilia olfactoria on the left hand side of the vesicle (arrows).

Figure 10. Cross-sectioned olfactory vesicle of cat III. Olfactory epithelium. Type II. Magnified 10,000 ×. The vesicle rests upon the supporting cell projections. The 8 basal bodies are interconnected by electron-dense lines lying in the vesicle.

Figure 11. Oval olfactory vesicle of cat II. Olfactory epithelium. Type I, 10,000 ×. From the intra-epithelial part of the dendrite provided with filiform structures a fibre bundle emerges, which divides into at least three small bundles (arrow). Each of them proceeds to a basal body. From the basal body an olfactory hair protrudes and immediately runs parallel to the surface.

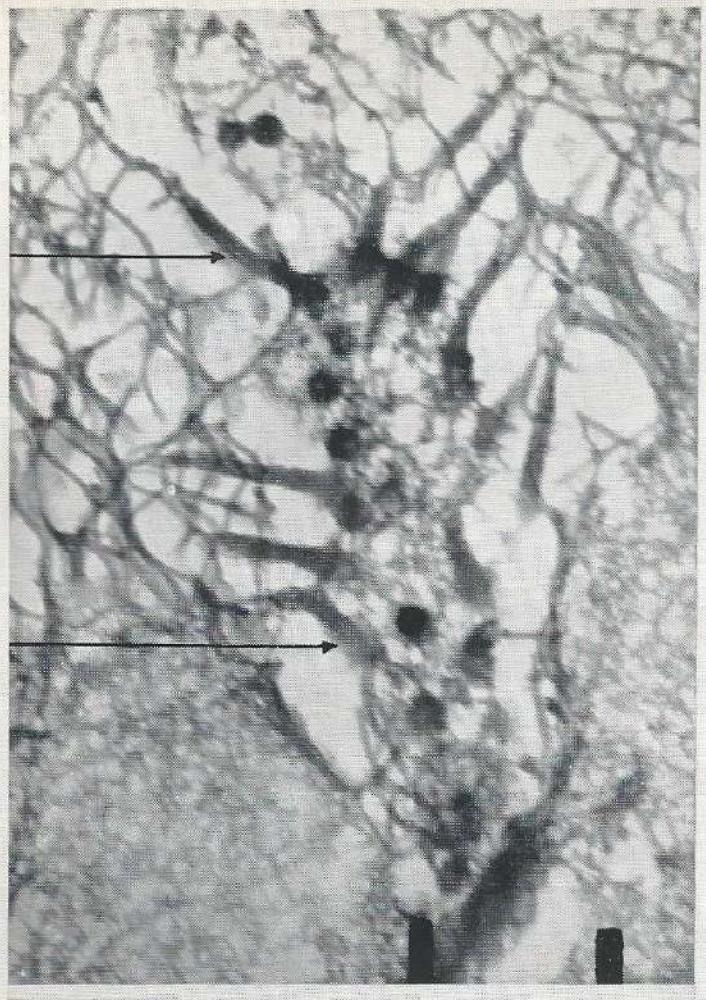


Fig. 9

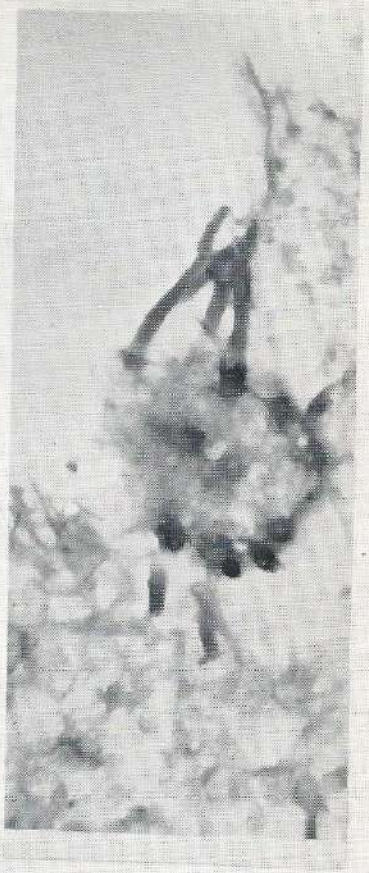


Fig 10

Fig. 11

two nucleoli. Either basally, as in the cat (figs. 2, 3 and 4) or throughout their cytoplasm, as in man, (fig. 15) these cells are full of granules. Electron-dense structures were found to be absent in the upper part of the cell in the cat (figs. 4 and 5) as well as in man (fig. 40). In a cross-section of the cat's olfactory region, we see a distinct system of intracellular concentrally arranged membranes, (fig. 6) especially in the middle layers near the nucleus. Whenever there are many granules, the membranes are pushed to the periphery of the cell, as observed in man (fig. 15). In the cat, however, some granules are also found between the membranes (fig. 6). Whenever the superficial elements are cross-sectioned we see opaque ring-structures lying in a circle, connected by a membrane (fig. 40).

In a longitudinal section (fig. 3 and 15) we see longitudinally directed fibrils. The fibrils are either connected with or lie next to large numbers of granular structures and pigment granula, the latter being most numerous basally in cat's epithelium. The infranuclear, centrally, electron-dense part of the supporting cell, together with its fibrils, divides into branches and extends between the basal cells. Here, too, many pigment granula are found, lying in rows in the branches. These branches appear to be intermingled with the axons of the olfactory cells, in this way forming a network as described by EXNER (1871). This infranuclear part has a cytoplasm which seems to consist of a dense, apparently amorphous, intermediate substance, within which can be seen darker granules. The supranuclear, wide portion contains a Golgi-network around the nucleus: a system of membranes and vacuoles of varying size which occasionally are also visible infranuclearly. From the most peripheral part of the supporting cell protrude protoplasmic projections. In the olfactory epithelium with a cuticula the tops of the supporting cells end in the syncytial membrana olfactoria limitans, covered with the protoplasmic projections. The vacuoles, which in the present study are mostly found in the peripheral part of the cell, are lined with filiform projections similar to, but smaller than those on the surface (fig. 16). In a vacuole lying near the surface small discontinuities of the covering cuticular cell membrane ($\pm$ 800 Å thick) are visible, seemingly gaps, but covered by a thin membrane ($\pm$ 100 Å thick).

§ 5. THE PIGMENT. In the adult cat (cats I—V) the pigment is mostly found at the base of the epithelium, where it appears in rows in the branchings of the supporting cells, the spaces between them containing olfactory axons (fig. 2). In the cat the pigment granules could rarely be found in the periphery. In normal human olfactory epithelium (homo I) granules occur throughout the whole of the supporting cell; especially around the nucleus (fig. 15) there are large numbers of them. In the cat pigment was also found in the subepithelial tissue within the deeply lying basal cells. Here a great many of inactive pigment granules are found arranged around the nucleus.

Finally the Bowman gland cells, which are discussed further on in this chapter, may contain larger numbers of pigment granula than the supporting cells. Very few pigment granules are found in young cats, (cats VI—IX), young humans (Homo II) and in pathological olfactory epithelium (cats V and XIV, Homo III). The pigment granula

Figure 12. Olfactory vesicle of cat XII. Magnified 40,000 ×. On the left and the right upperside of the vesicle one finds a longitudinally sectioned cilium olfactorium containing a bundle of one central fibril and on each side a peripheral one of the nine paired peripheral fibrils, all of which can only be distinguished on cross-section. All these fibrils proceed via the basal body to the interior of the vesicle (arrow 1). The fibrils are surrounded by a thin, fragile sheath, which merges into the cover of cuticular protoplasm of the vesicle (arrow 2). In the basal body an electron-dense cross-plate is present, through which the fibrils proceed into the centre of the vesicle. In the lengthening of this cross-plate is visible an electron-dense linear structure interconnecting two basal bodies (arrow 3).

Figure 13. Olfactory vesicle of cat II, cut tangential to the lateral side of the vesicle and parallel to the axis. Magnified 20,000 ×. We can see the angle (120°—145°) which the fibril bundle (arrows) proceeding from the vesicle through the basal body, makes with the ascending fibrils of the olfactory hair on the lateral side of the vesicle. The electron-dense cross-plate in the basal body forms the connection between both fibril bundles.

Figure 14. Olfactory vesicle of cat II, cut tangential to the upper-lateral side of the vesicle. Magnified 5000 ×. A thin cover of cuticular protoplasm around the vesicle, which cover merges into the thin sheath around the cilia olfactoria, is visible (arrow 1). Within the basal body the basal cross-plate (arrow 2) is visible. In the lengthening of this cross-plate the interconnecting structure lies among the basal bodies.

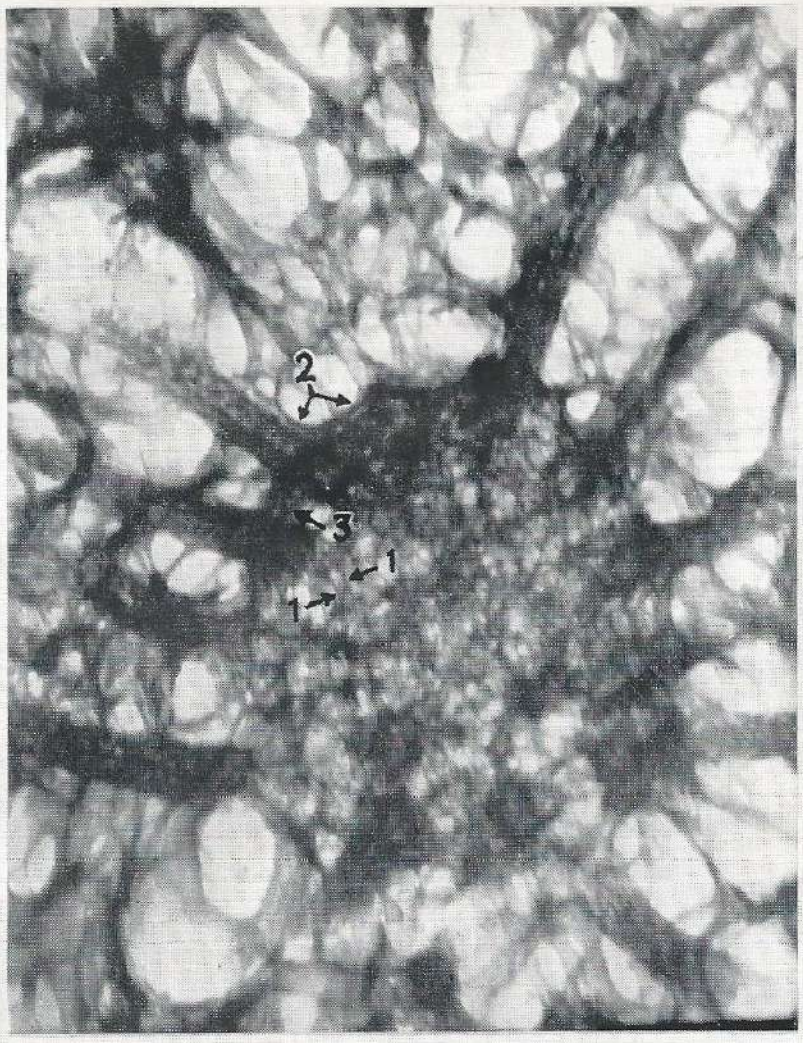


Fig. 12

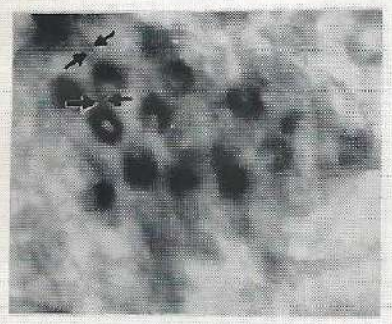


Fig. 13

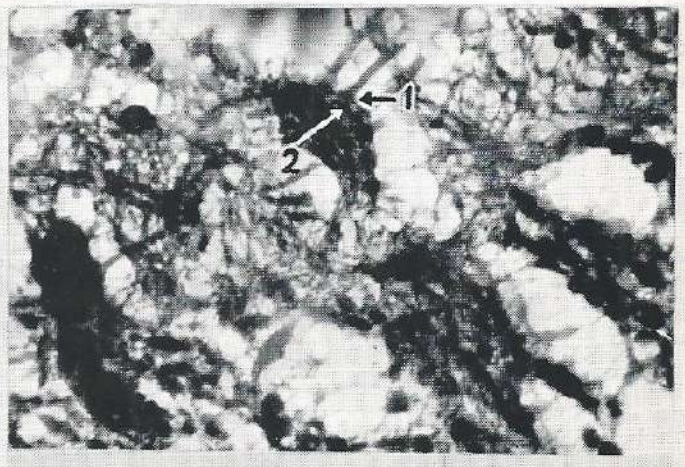


Fig. 14

in a supporting cell of human olfactory epithelium are of different sizes. The small ones lie in less opaque spaces which are of the same form and size (1—1.5 μ) as the large granula (fig. 15).

§ 6. MEMBRANA LIMITANS OLFACTORIA OF VON BRUNN. A thin translucent border can be seen with the light microscope. In the electron microscope, in the olfactory epithelia described firstly and secondly, it appears to consist of hair-shaped projections from the supporting cells. These projections form a sort of network which supports the olfactory vesicle and their cilia. The thin sheath around the cilia olfactoria merges into a thin cover of some kind of cuticular protoplasm around the vesicle (fig. 19 arrow 1). The electron-dense borders limiting the protoplasmic projections of the supporting cells also merge into the opaque borders limiting the surface of the supporting cells (fig. 19 arrow 2). So the whole surface structure is covered with a sort of cuticular border, $\pm$ 200 Å, sometimes 800 Å, in width on top of the supporting cells. Nevertheless, besides these hair-shaped projections and the olfactory vesicles with their limiting membranes a real olfactory limiting membrane is found on the olfactory epithelium. This cuticula, a syncytial reticular membrane, covers the entire surface, except in a few accidental areas where the supporting cells are provided with the protoplasmic projections only. It forms superficial club-shaped and cauliflower-like swellings, from which tubular projections arise similar to those seen on the supporting cells (fig. 17).

Downwards a network of partitions is formed between supporting cells and olfactory cells. Sometimes a small projection extending from a partition into a supporting cell is seen (fig. 18 arrow 4). Such a projection may occasionally enclose small parts of the supporting cell (fig. 6, arrow 4). A partition completely or almost completely surrounds both the peripheral dendrite and the olfactory cell perikaryon and only partially surrounds the central axon. Pigment granules are found in the basal part of the partitions consisting of the very osmiophilic branches of the supporting cells. The peripheral olfactory dendrites thus run through canals which are either entirely or partly closed. Those canals lie in the partitions as well as in the cuticula itself. The partitions surrounding the perikaryon of the olfactory cell can also be connected with the excretory ducts of the Bowman glands (fig. 18 arrow 5 and drawing 3). In this way these excretory ducts can be surrounded by some olfactory cells and their nuclei. This cuticula and these partitions are composed of a dense, apparently amorphous, osmiophilic substance within which can be seen darker granules (fig. 17). The cell-membranes of both the olfactory cells and the supporting cells lie outside the granular structure of the partitions (figs. 7 and 15).

§ 7. HAIR-SHAPED STRUCTURES ON THE SURFACE. From the supporting cells protrude protoplasmic projections. These projections are usually broken off but if handled carefully we find they have a length of 3 μ with a diameter of 0.05—0.1 μ. A large number of them is seen on each cell. A transverse section reveals them to be without fibrillar structure and without basal bodies, but both on cross and longitudinal section they seem to be tubularly bounded by a dense membrane (fig. 17 and 19) that merges into the opaque borders bounding the surface of the supporting cells (fig. 19 arrow 2). The

Figure 15. Supporting cell of human olfactory epithelium Homo I. Magnified 10,000 ×. Large amounts of pigment granules surround the nucleus. Here and there the granules are very small, indistinct or divided in two or more parts (arrow 1), partly deliquesced and lying in bigger spaces. To the left of the nucleus a few mitochondria-like structures are visible. At the bottom to the left an intercellular partition (arrow 2) lies next to the peripheral portion of the olfactory cell that is provided with protofibrils (arrow 3). The longitudinally directed membranes and circular structures of the endoplasmic reticulum are pushed to the periphery of the cell (arrow 4). The distal portion of the cell is destroyed.

Figure 16. Olfactory epithelium of cat V, uppermost portion of the supporting cell. Magnified 10,000 ×. At the innerside the vacuoles are covered with small filiform projections (see especially arrows 1) smaller than the projections on the surface of the cell. At the surface we can see small gaps of the cuticular cell membrane (arrows 2) closed by a very thin membrane (100 Å in diameter). A part of the surface of the cell is covered with a cuticular structure, ± 800 Å in diameter.

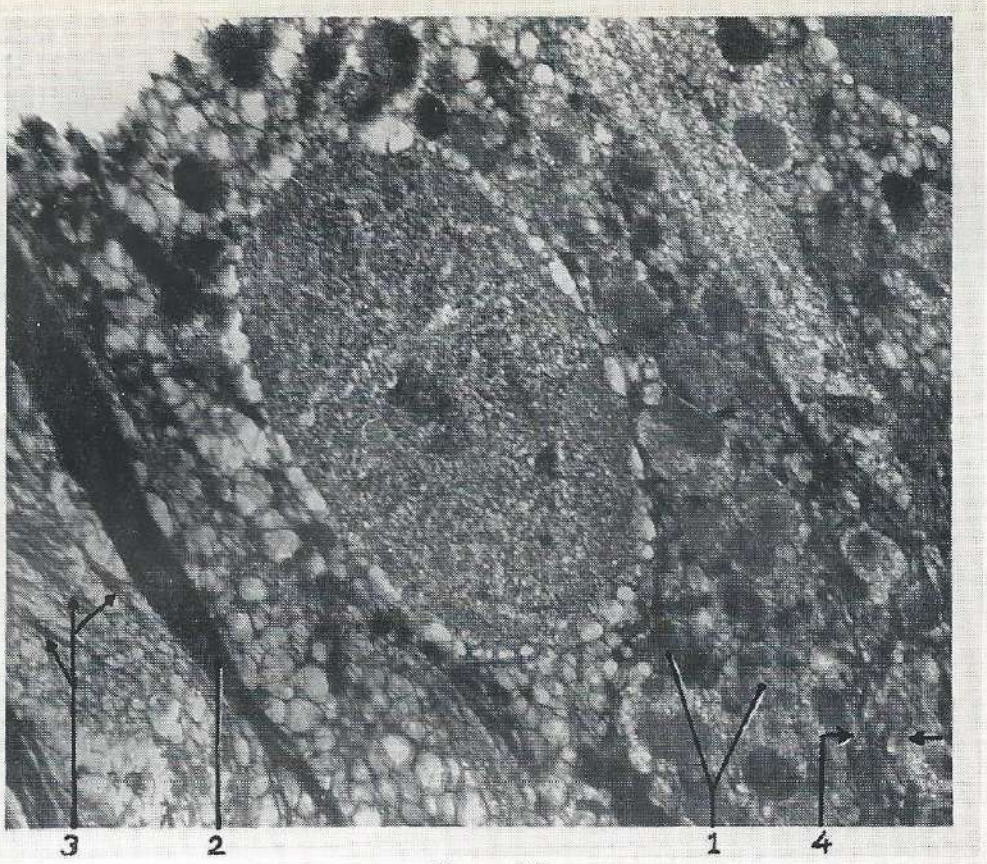


Fig. 15

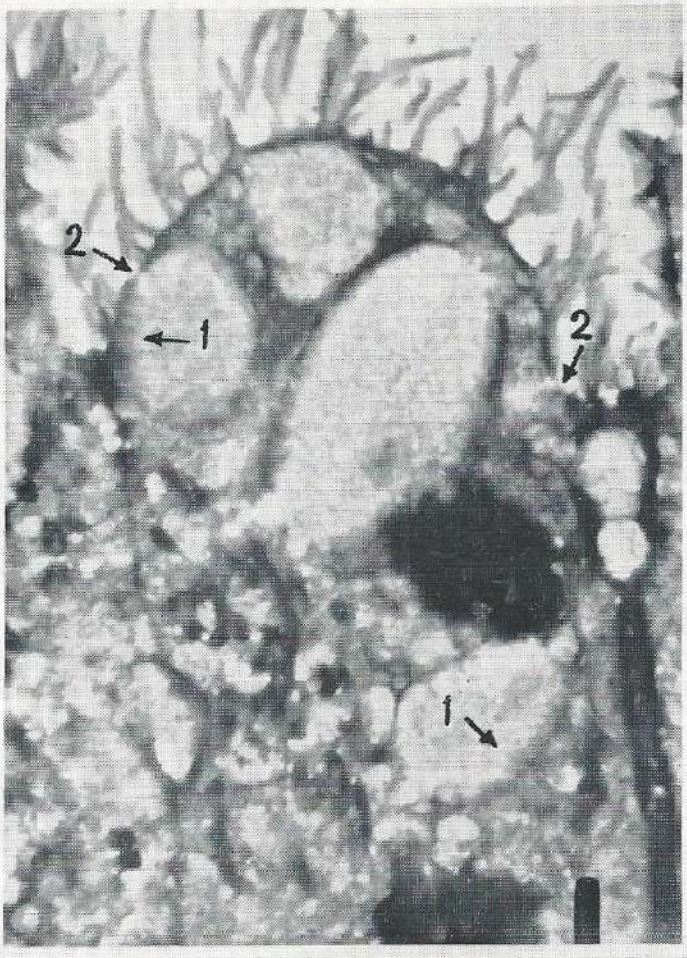
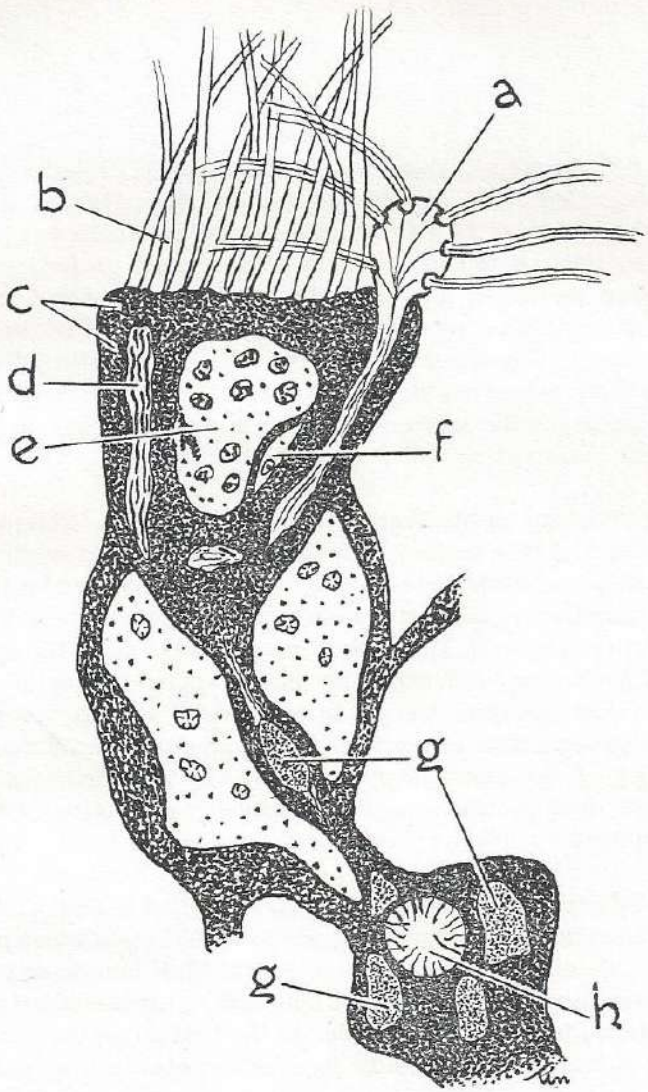


Fig. 16



Schematic drawing 3 (compare with fig. 18). Oblique section of the membrana limitans olfactoria with its partitions and their canals, in which parts of the olfactory neurons containing electron-dense thread-like structures are visible. The partitions are connected with the excretory duct of the Bowman gland in which small protoplasmic projections protrude. This duct is surrounded by some olfactory cells and their nuclei; a = vesicula olfactoria, b = tubular protoplasmic projections, c = cuticula with its partitions, d = olfactory dendrite, e = supporting cell, f = projection of a partition in which some supporting cell protoplasm is enclosed, g = olfactory cell nuclei, h = excretory duct of the Bowman gland.

Figure 17. Olfactory epithelium of cat XII. Type III. Membrana limitans olfactoria. Magnified 5000 ×. Oblique section. Superficially is seen the granular cuticula (arrow 1) with its club-shaped or cauliflower-like ends of the supporting cells on top of which the hair-shaped projections seem to have a tubular character. The cuticula consists of a dense, apparently amorphous, intermediate substance, within which can be seen darker granules. Arrow 3 cilia olfactoria. Arrow 4 an intra-epithelially situated olfactory dendrite cross-sectioned. The arrow 2 indicates a cut through a vesicle. Underneath the surface vacuoles are present within the peripheral processes of the supporting cells.

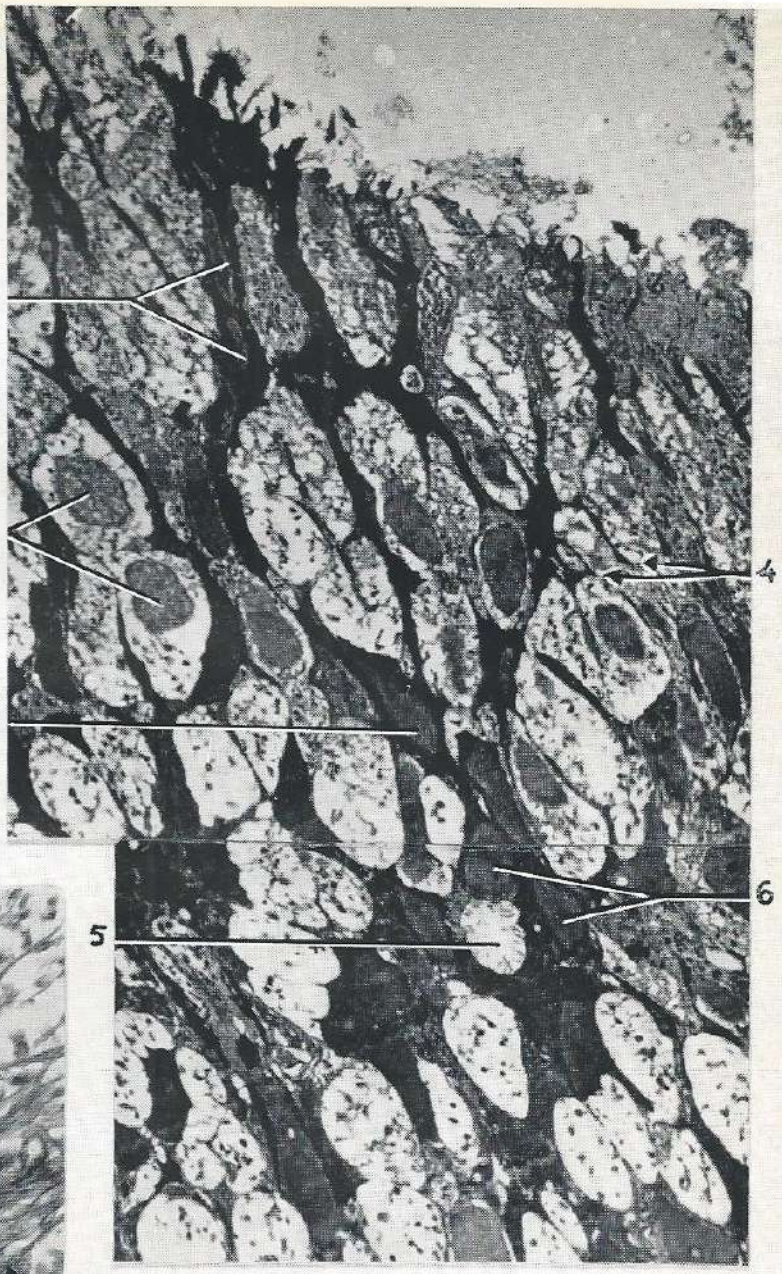
Figure 18. Olfactory epithelium of cat II. Type III. Oblique section. Magnified 3000 ×. At the surface, club-shaped or protracted swellings arising from the partition network and olfactory membrane. Just under the surface there is a connection visible between several partitions whose tiny canals contain dendrites (arrow 1). The supporting cell nuclei lie in the open spaces (arrow 2). The olfactory cell nuclei lie mostly embedded in the partitions (arrow 3). Projections from the partitions (arrow 4) into the supporting cells are also present. The connection of the olfactory membrane with the excretory duct of Bowman gland (arrow 5) via the partitions is clearly visible. In this duct protrude small protoplasmic projections. The duct is partly surrounded by olfactory cells and their nuclei (arrow 6).

Figure 19. Olfactory epithelium of cat XII. Magnified 15,000 ×. A network of tubular projections of the supporting cells is visible, upon which projections a vesicula and its cilia olfactoria with a central fibril bundle rest (top left). The tubular structure of the projections bounded by a dense-edged membrane (on cross-sections, too) is clearly visible. At the bottom on the left a vacuole bounded by a long mitochondrium in which cristae mitochondriales are visible.

Arrow 1. The thin granular sheath around the cilia olfactoria merges into the thin covering protoplasm around the vesicle.

Arrow 2. The electron-dense membrane bounding the tubular protoplasmic projection is continuous with the opaque membrane bounding the supporting cell surface.

Fig. 18



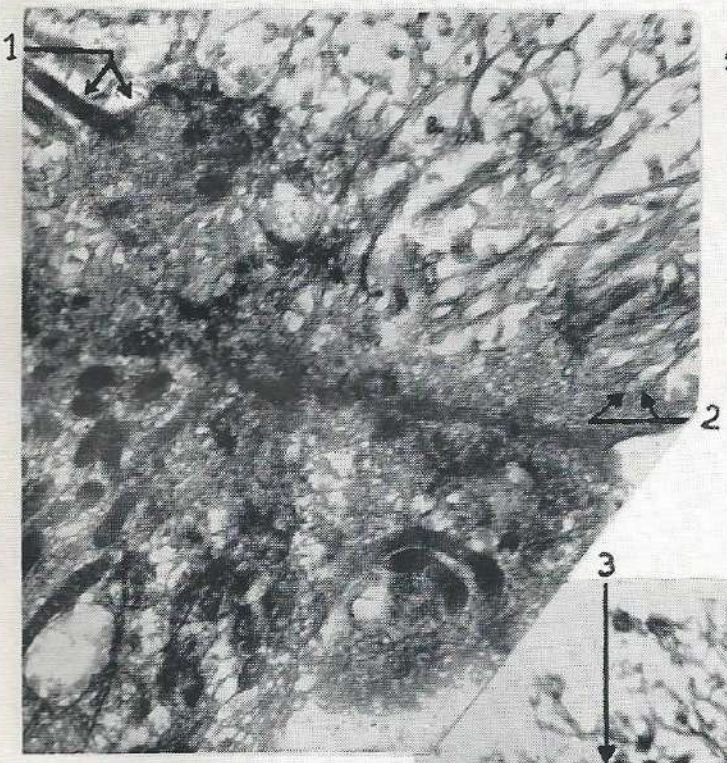


Fig. 19

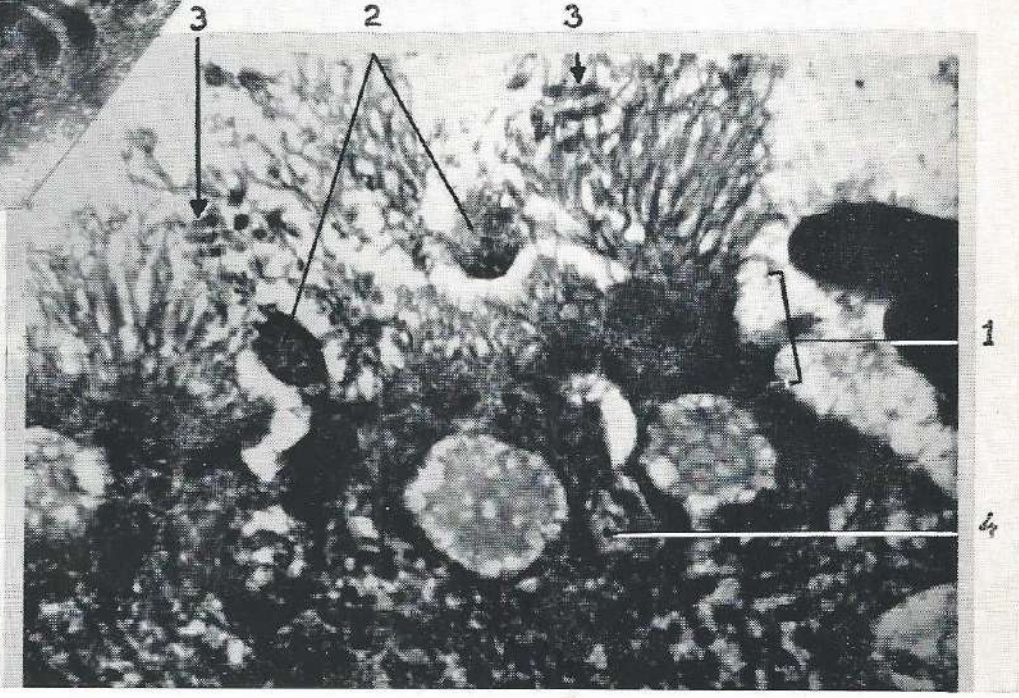


Fig. 17

projections seem to form a branching network, but on closer examination they prove to be only interlaced. Their base is somewhat wider and they taper towards the top.

The second kind of the hair-shaped structures at the surface of the olfactory epithelium are the cilia olfactoria. Most of them seem to be broken off and in our preparations are from 2—3 μ in length. They rest upon and lie between the network of supporting cell projections and have no connection with these projections. They protrude from a basal body half on top of and half within the olfactory vesicle. This may lead us to assume the presence of structures similar to the cilia of the uppermost respiratory tract mucosa: an axial fibrillar bundle surrounded by a thin peripheral granular sheath; this sheath merges into the covering cuticular protoplasm around the vesicle (fig. 19). The axial bundle in these cilia consists of nine paired fibrils in the outermost layer and two in the centre. This axial fibrillar bundle with all its fibrils penetrates through the basal body which contains an electron-dense cross-plate. Inside the vesicle this fibril bundle joins bundles from other cilia. The cross-plates of the basal bodies are interconnected by an opaque structure only visible on cross-sections of the vesicle (fig. 12 and 14).

§ 8. THE REPLACEMENT OR BASAL CELLS. Some basal cells that sometimes lie subepithelially are provided with pigment granula around their nuclei. Intra-epithelially the basal cells can contain axons surrounded by double membranes. These cells will be discussed extensively in part II in connection with the regeneration process.

§ 9. THE GLANDS OF BOWMAN. In the cat (fig. 32) and in man (fig. 20) the subepithelial tissue remotest from the olfactory epithelium may contain acini consisting of indifferent cells. There is more than one row of cells around the lumen and the cells overlap (fig. 20). In the cells few mitochondria and some tiny granula are visible around small spaces. These gland cells are very numerous in abnormal olfactory epithelia (Homo II and cat VII).

In normal epithelia (cats III, IV, X, Homo I) ducts surrounded by gland cells with large pigment granula lie in the deepest layer of the subepithelial tissue. Among them are found a few acini consisting of small indifferent cells and several ducts consisting of gland cells full of secretion spaces. The cells containing granula and lacking secretion spaces lie around flattened ducts; they have nuclei with a mosaic-like chromatin structure (fig. 22). The cells with secretion spaces occur throughout the subepithelial tissue, they are elongated in shape; on cross-section they prove to lie around the lumen, so the gland exhibits an acinous form (fig. 21). The nuclei are round and lie in the middle of the cell; the cells contain a large number of granules (fig. 22 and 23) with a maximum diameter of 1—1½ μ, when not surrounded by secretion.

Mostly these granula lie in the middle of their secretion spaces. Smaller granula lie excentrically within the secretory space (fig. 23, arrow 1); the remnant of the granulum seems to be in close contact with the separating membrane (fig. 23, arrow 2).

The secretion cavities are separated by membranes which seem to be covered or to be in close contact with several spherical remnants of the secretion granula protruding into the cavities. In the apical portion of the cell, the old secretory cavities can be recognized as small spaces either or not filled with secretion and surrounded by pin-

Figure 20. Olfactory epithelium Homo II: (a 9-month-old child). Magnified 2000 ×. Bowman gland with small indifferent cells without pigment granula and with few mitochondria. Some cells protrude into the lumen (arrow 1). At the top on the left a gland-like structure is seen, small intracellular cavities surrounded by tiny osmiophilic granula (arrow 2). There are no mitotic structures visible. Two or three cell layers surround the lumen.

Figure 21. Human olfactory epithelium. Homo I. Magnified 2000 ×. Secretory gland of Bowman at about the final stage of its secretion. Pin-point-like osmiophilic granular structures are found along the separating membrane of the secretion cavities. More towards the lumen, we see small spaces, mostly empty, sometimes filled with secretion surrounded by these pin-point-like granular structures. The gland cell is covered with a brushlike border. Under this border on the cell membrane also lie some tiny pin-point-like granula. The cell is elongated and the nucleus is round and lies in the centre of the cell. At arrow 1 there is present an intercellular canal containing hair-shaped protoplasmic projections protruding from the gland cells. There are some small gaps of the cell membrane on the lumen side (arrow 2) and on the side of the intercellular canals. These gaps, however, are covered with a very thin membrane.

Figure 22. Olfactory epithelium of cat III. Magnified 3000 ×. Gland of Bowman. The broadened gland cell contains ocellus-like secretory granules 1—1½ μ in diameter not surrounded by secretion. The nucleus exhibits mosaic chromatin structures. The flattened lumen contains hair-shaped protoplasmic projections. At the top on the left, a gland cell with products of degeneration in process of desintegration.

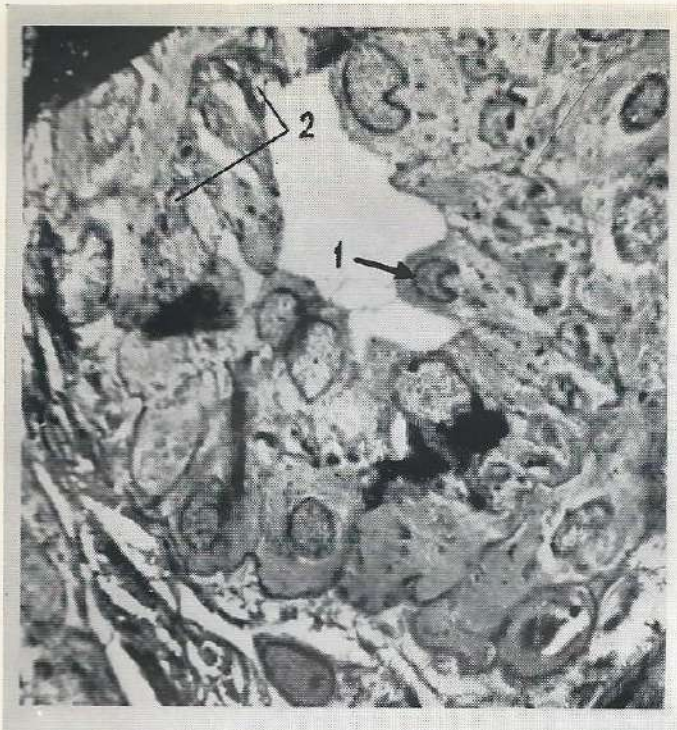


Fig. 20

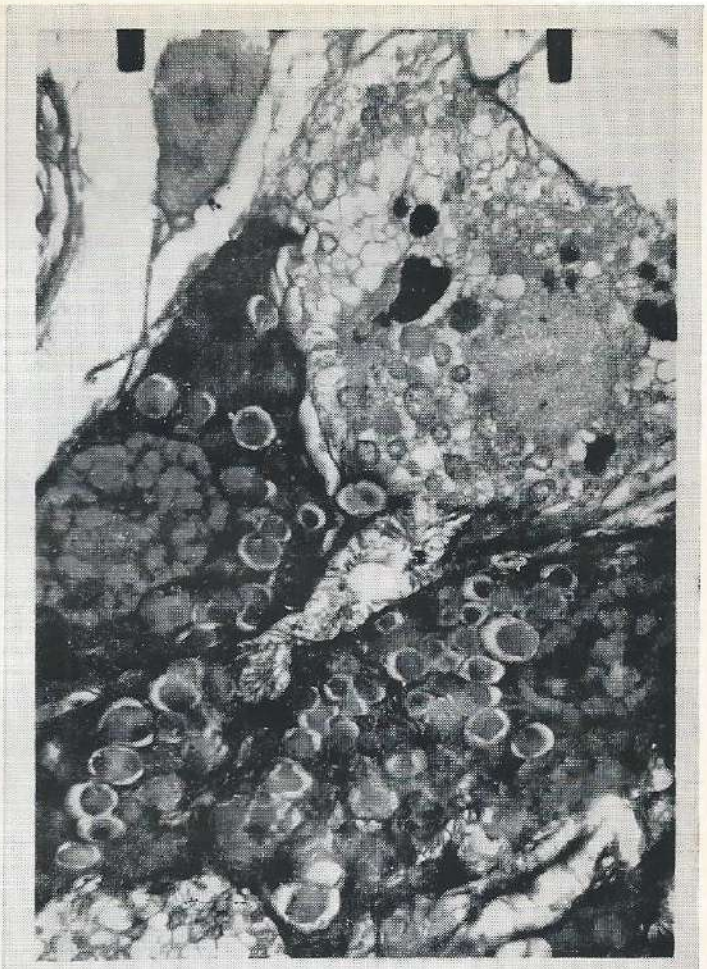


Fig. 22

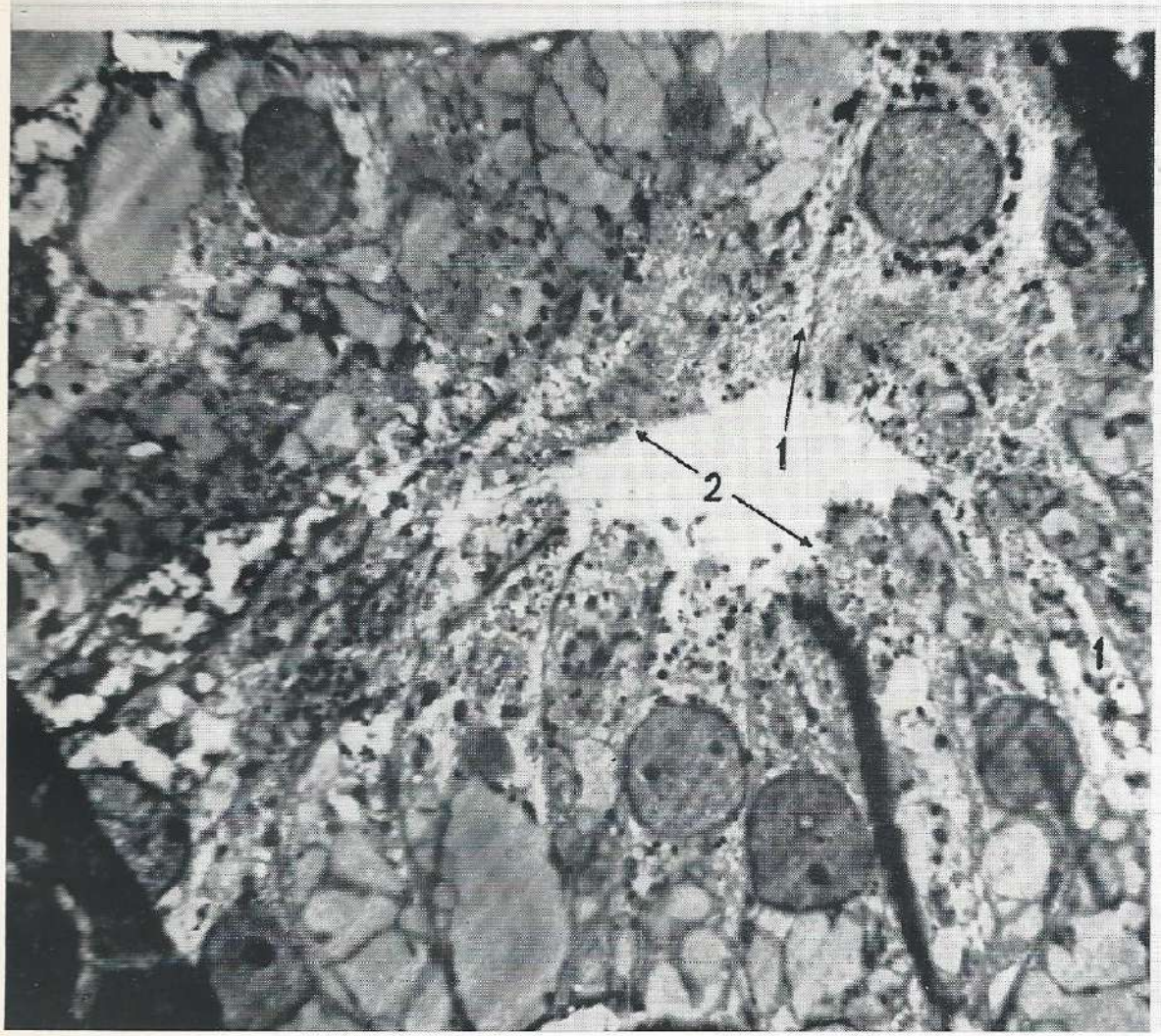


Fig. 21

point-like granular structures (fig. 21). Intercellular canals with tiny protoplasmic projections occur (fig. 21, arrow 1). Small seeming discontinuities of the cell membrane on the lumen side and also on the side of the intercellular canals (fig. 21, arrow 2) are visible. These discontinuities however, are covered with a very thin membrane.

The surface of the gland cells, the ducts, and the tiny intercellular canals, are covered with small hair-shaped projections (1—1½ μ in length and 0.03 μ in diameter) which are interlaced. The lumen of the ducts is sometimes partially filled with these interlaced projections which seem to be tubular on longitudinal section (fig. 25). Underneath the olfactory epithelium the glands are tubular and their cells at rest, containing some secretion rests (fig. 24). The small areas mostly empty, sometimes filled with secretion, surrounded by pin-point-like granular structures are still distinct (fig. 24 arrow 1). Moreover, the varying degree of osmiophilia of the nuclei, not so distinct in the other stages, is noteworthy. More and less opaque nuclei are clearly seen here. In this stage the gland ducts lie close underneath the olfactory epithelium and at places encroach upon it (fig. 2). These gland cells we see in greater numbers in abnormal olfactory epithelia (Homo II, cats VI and VIII).

Sometimes a gland cell degenerates, which may happen in each stage. In man as compared to cat the somewhat thicker basal membrane around the duct is more striking (fig. 20 and 24). The gland duct entering the epithelium may be surrounded basally by gland cells, but in cat III somewhat higher in the epithelium the duct is surrounded by olfactory cells (fig. 18 and 25). In cats II and III the excretory duct is also surrounded intra-epithelially by supporting cells (fig. 4 and 26). In cat III these olfactory cells as well as these supporting cells may be connected with the partition-network of the olfactory membrane (fig. 26).

Finally, in cat III and XII, the gland drains at the surface into small crypts covered with tiny hairs (1—1½ μ in length, 0.03 μ in diameter. The small-type excretory duct hairs predominate at this place over the larger-type supporting cell hairs (3 μ in length, 0.05—0.1 μ in diameter).

Figure 23. Olfactory epithelium of cat IV. Magnified 5000 ×. Active secretion in duct of Bowman gland in longitudinal section. In the beginning of the secretion the granulum lies in the centre of its secretory space and later on excentrically (arrow 1).

The remnant of the granulum seems to come into close contact with the separating membrane (arrow 2). The secretory product lies upon the protoplasmic projections protruding from the gland cell surface (arrow 3). At the top on the left a nucleus of the gland cell.

Figure 24. Gland of Bowman at rest. Olfactory epithelium (Homo II, a 9-month-old child). Magnified 3000 ×. The various moderately and more osmiophilic nuclei are striking. The tiny areas, lacking secretion, at the time of the investigation, are surrounded by point-like tiny granula (arrow 1). At the bottom on the right there are still several remnants of secretion-spaces each containing a small deliquescing granulum (arrow 2). A thick basal membrane surrounds this entire gland portion. At the left upperside a blood capillary filled with a leucocyt and some erythrocytes (arrow 3).

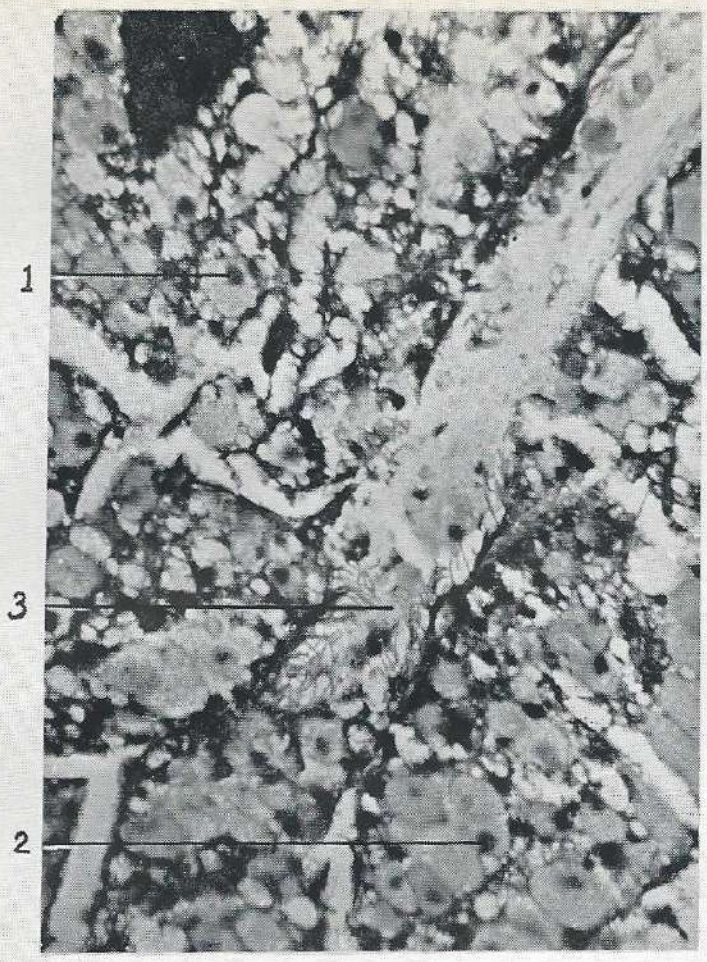


Fig. 23

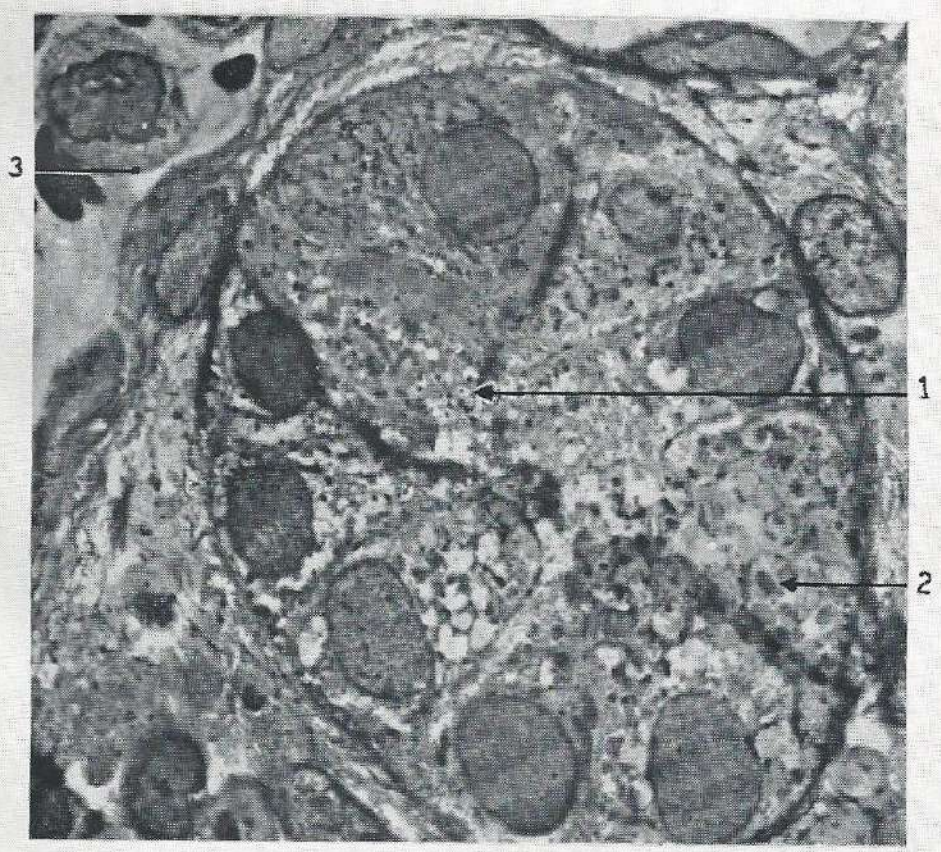


Fig. 24

CHAPTER IV

COMMENT

§ 1. TYPES OF OLFACTORY EPITHELIUM. Three types of olfactory epithelium are found in the present study. In the commonly known type also described in light-microscopic studies, here called type I, the vesiculae olfactoriae lie above the surface of the supporting cells. The superficial protoplasmic layer devoid of nuclei is bounded below by a layer of supporting cell nuclei. Basal cells are practically absent. This type is found in cats II, IV, X, XII. As these cats have neither pus nor mucus in their nasal cavities they may be supposed to have normal olfactory epithelium.

In type II found in cats III, XI and XIII the vesiculae olfactoriae are found within the cavities formed by the walls of the supporting cells. The uppermost row of nuclei are the olfactory cell nuclei and not the supporting cell nuclei, as in type I. Under the row of supporting cell nuclei an enormous number of basal cells is visible.

The existence of the membrana olfactoria limitans of VON BRUNN is confirmed by the fact that a cuticular structure has been found in cats III, IV, X, XII. Moreover there exists a large network of partitions between the supporting and the olfactory cells, which network originates from the membrana olfactoria limitans. This olfactory epithelium is called type III. This cuticula and the partitions both seem to consist of a dense apparently amorphous substance, within which can be seen very small dark granules.

ENGSTRÖM, BLOOM (1954) and others have seen these granular structures in lipoid-containing membranes. Since VON BRUNN only found this structure after prolonged osmic acid impregnation, the membrane and the partition network were possibly washed away in the other types. Nevertheless, after prolonged fixation of cat X's olfactory epithelium with osmic acid we found the other two types too. Increasing the fixing-time by 2—3 hours, however, produced preparations with a network of slender partitions ($\pm$ 0.2 μ in diameter) lying between the supporting cells just under the surface and very much thinner than the one shown in the picture of type III ($>$ 1 μ in diameter) (fig. 6 and 7); a cuticula, however, was not seen in types I and II olfactory epithelium. In the cat type III olfactory epithelium was found only in the posterior part of the nasal cavity. According to ADRIAN (1950, 1956), volatile oils produce more action potentials in the posterior pole of the bulbus olfactorius. In the cat there appears to be, posterior to this region, an area which gives off more action potentials when the animal smells fish or meat (amines in decomposition). From this he concluded that the olfactory organ was not a uniform mosaic of receptors.

Figure 25. Olfactory epithelium of cat III. Type III. Magnified 20,000 ×. Excretory duct of Bowman gland. This excretory duct is commonly surrounded by fibres, in this epithelium, however, also by the partitions originating from the olfactory membrane. The ocellus-like granules in the partitions are distinct. On the left an osmiophilic olfactory cell nucleus. The lumen surface is covered with little tubular protoplasmic projections (± 1 μ long). On the left side the inner surface of the duct is destroyed and therefore the lumen directly borders on the olfactory cell nucleus double membrane.

Figure 26. Olfactory epithelium of cat III. Type III. Magnified 5000 ×. Excretory duct of Bowman gland, the walls of which are formed by supporting cells whose nuclei border on the lumen of the excretory duct (the surface of the cell is partially destroyed there). The excretory duct with the supporting cells is enclosed by the electron-dense central portions of the supporting cells (arrow 1). At the top on the right an intercellular partition (arrow 2) with dark granula is connected with a supporting cell (arrow 3).

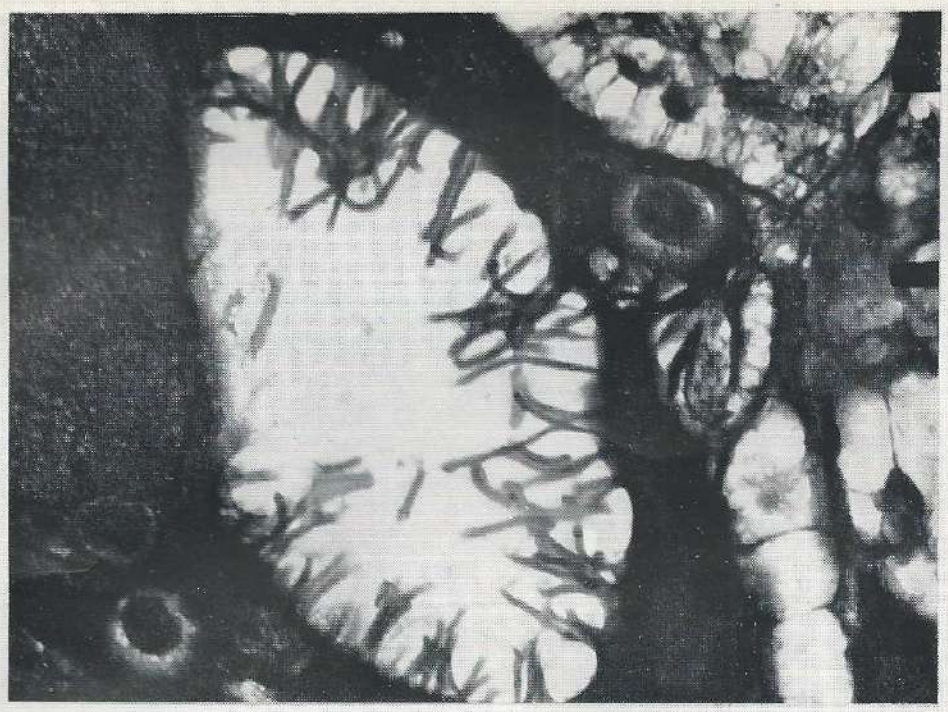


Fig. 25

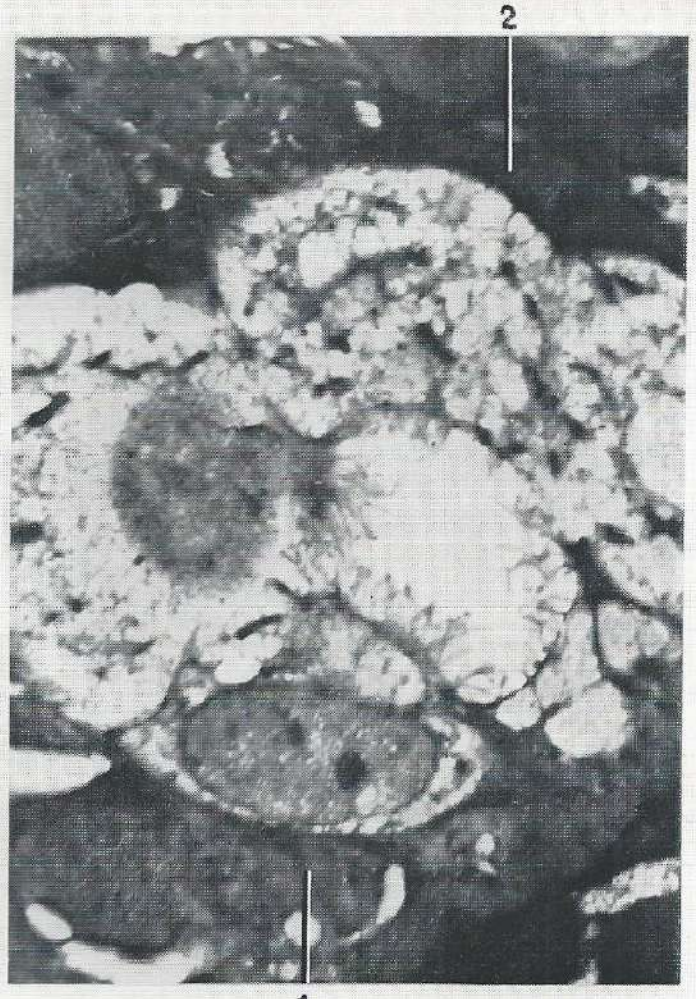


Fig. 26

LE GROS CLARK and WARWICK (1951), by systematically destroying one section of the bulbus olfactorius at a time and exploring the whole of the olfactory epithelium, showed that there was a topographic projection of the superficial neuro-epithelium on the glomeruli of the bulbus, viz.: an anterior-posterior and a superior-inferior projection. There seemed to be no median-lateral projection. Neither of them could find a point-to-point projection, which is conceivable when we consider the interconnections of the neurons, as described above. From these investigations of ADRIAN and LE GROS CLARK and from our own work, it appears that probably type III epithelium with the membrana olfactoria is the one stimulated by volatile oils and/or meat and fish. In superior-inferior direction no different morphology has been found.

In the posterior part of the cat's nasal cavity basally in the epithelium we also found large quantities of pigment in both the supporting cells and the Bowman glands. The greater sensitivity and higher resistance against exhaustion in those parts with great amounts of pigment, as found by ADRIAN, can be correlated with the greater number of action potentials found by him in the posterior region of the bulbus of the cat.

In man the membrana olfactoria was not found, presumably because it was impossible to obtain a complete section of a fresh and undamaged human olfactory epithelium. Here the partitions were seen more basally.

§ 2. THE OLFACTORY CELL BODY. In a perpendicular section the olfactory axon is mostly cut only partially or not at all. (fig. 3). This may be due to two reasons:

1°. The central process has a smaller diameter than the peripheral one (this is the only cause given by GASSER, 1956).

2°. The central process of the nerve cell may form an angle with the long axis of both its nucleus and its peripheral process, which is the opinion, that we favor.

Contrary to other authors, we observed interconnections between the axons of the cells (fig. 6). The electrical activity derived from the olfactory epithelium, led ADRIAN (1956) to suppose that there must be some sort of co-operation between the receptor cells. Maybe the interconnections can account for such a co-operation.

SJÖSTRAND and ENGSTRÖM (1954) stated, that in the cochlea, the acoustic terminals originating from the spiral ganglion and ending basally to the inner- and outermost haircells, contained some mitochondria. They did not find any mitochondrial structures in the peripheral neuronal endings lying just under the haircells. Analogous to these findings we could not find any electron-dense structure either in the terminal part of the olfactory dendrite. In analogy with what HARTMAN (1953) a.o. saw in axons, we found in the central and peripheral portion of the olfactory cell electron-dense filiform structures in whose lengthening the protofibrils lie. These filaments also seem to be conglomerations of the protofibrils (fig. 8). They look like mitochondria, but they exhibit a longitudinally striped appearance. A number of mitochondria are yet seen in the dendrite, but they are not always arranged in two levels as described by BLOOM (1954) and GASSER (1956).

§ 3. THE VESICULA OLFACTORIA. As we have pointed out in type II epithelium of the cat, where the vesicles lie within depressions, the vesicles do not always protrude 2 μ above the surface as BLOOM (1954) asserts. Different sizes of the vesicles were also

described by LE GROS CLARK (1946), but not different shapes. We have found very small vacuoles and some lipoid granules in the centre of the vesicles. These granules also described by BLOOM, appear to be the basal bodies of the cilia (fig. 9 and 10). We have also found a thin cover of cuticular protoplasm on top of the vesicles as described by ENGSTRÖM (1952). This cover merges into the thin sheath around the cilium, as we pointed out. Contrary to the findings of BLOOM and others who report a maximum of 12 cilia olfactoria in the frog and 16 in the rabbit (LE GROS CLARK), we have seen in cat 36—40 cilia on one vesicle.

According to BLOOM the cilia proceed over a short distance perpendicularly towards the surface, then bend and run parallel to it, forming a dense network. In the present study this course is seen in the cilia of the vesicles lying in depressions. The cilia olfactoria of the vesicles lying beyond the surface of the supporting cell curve immediately and run parallel to this surface, as we see in fig. 11. In the preparations, apparently at about 1.5—3 μ from their basis, the cilia are either broken off or dissolved during fixation and staining, or possibly they run in another direction and therefore the distal segment is not included in the section. The inner structure of the common cilia was already described by ENGSTRÖM (1952) for the tracheal cilia and by FAWCETT and PORTER (1953) for all sorts of cilia in both animals and plants. The olfactory cilia inner structure was described by BLOOM (1954) in the frog. Contrary to GASSER we did not see a cilium divide into long thin ribbons to a length of 100 μ. Neither BLOOM nor ENGSTRÖM could find the basal bodies of the cilia olfactoria in the frog and in man. We tried to find them in man, but all their vesiculae olfactoriae were destroyed by a variety of causes; we only found them in the cat.

§ 4. THE SUPPORTING CELL. In accordance with BLOOM we mostly found intracellular membranes near the nucleus (fig. 4 and 6). These cytoplasmic structures are similar to the lamellae found by DALTON (1951), who used the electron microscope, in liver cells, body chief cells of the stomach and exocrine cells of the pancreas. They also resemble the cytoplasmic strips that MONROE (1953) found in the thyroid cells. SJÖSTRAND (1953) has elaborately described the membranes in the cells of the pancreas and of the tubuli contorti of the guinea pig's kidney. PALADE (1954) described a membranous system in 40 different cell types. ENGSTRÖM and WERSÄLL (1953) described these structures in the inner haircells of the organ of Corti. Moreover, WERSÄLL (1956) found the same structures in the haircells of the cristae ampullares. The supporting cell contains both circular and longitudinal structures of the endoplasmic reticulum. This was already described by GASSER. He was also the first to describe a large number of granules which push these structures to the periphery of the cell (fig. 15).

The "appareil reticulaire interne" observed by GOLGI (1898) in nerve cells, was originally described as a silver-stainable network. The "Golgi apparatus" has since been extended to comprise intracellular vacuoles and granules of extremely varying appearance and staining characteristics. According to PALADE, CLAUDE (1949) and others the myeline pictures of the Golgi apparatus are caused by progressive imbibition of the tissue during osmic acid fixation. These investigators report that the Golgi network can not be

demonstrated in living tissue, and consider it either an artifact or a malformation. In recent years, however, a Golgi apparatus has been demonstrated in living cells, among others by DALTON and FELIX (1954). Its existence as a true organelle may well be considered as generally accepted today. DALTON and FELIX (1954) in comparative studies of the Golgi apparatus both by light and electron microscope, observed an osmiophilic and an osmiophobic component. They found, that mitochondria and droplets in the epithelial cells of epididymis and duodenum of the mouse, supravitally stainable with either methylene blue or neutral red, had no morphologic relationship with the Golgi substance. SJÖSTRAND and HANZON (1954) studied the Golgi apparatus in the exocrine cells of the mouse pancreas. They found it to be divided into several intracellular zones. Each zone consisted of a system of membranes, vacuoles and granules of varying form and density. In our preparations, after fixation, a Golgi apparatus is found around the olfactory and supporting cell nuclei.

Within the supporting cells ALLISON, WARWICK (1947), LAMS (1940) and PLANEL (1951) found signs of secretory activity. We saw in cat V vacuoles lined with filiform projections. How these possible secretion spaces empty is not quite clear. Perhaps the secretion diffuses through the small gaps visible in the cell membrane covered by a thin membrane (fig. 16). Another possible way-out is provided by the protoplasmic projections at the cell surface, which occasionally may be covered with secretion. Possibly both routes are followed. The infranuclear central part of the supporting cell of both man and cat is branched and very osmiophilic. This part is so darkly stained that the axon is very difficult to distinguish. Its cytoplasm is of the same structure as the membrana olfactoria and it may be connected with the partitions (fig. 26).

In type II olfactory epithelium of the cat the upper part of the supporting cell, which does not contain electron-dense structures, exhibits a syncytial structure. In the same place the syncytial structure of the membrana limitans olfactoria is visible in type III olfactory epithelium. Intra-epithelially the olfactory cells with their axons are surrounded by supporting cells. Thus the latter cells apparently assume within the epithelium the rôle played subepithelially by the Schwann's cells; hence they possibly have some significance as supporting, isolating and nutrifying elements to the nerve cells.

§ 5. THE PIGMENT. Bloom described in the frog large amounts of granules diffusely dispersed throughout the supporting cell protoplasm; we observed the same in men. In the cat, however, we saw the granules only basally in the supporting cells. Here the granules are all of the same size, they are inactive and lie as it were in a depôt. In superficially destroyed human olfactory epithelium they seem to come to activity; just as in Bowman glands the granules may decrease in size and disappear till only a shadow is left, probably due to deliquescence of the pigment (fig. 15). So probably the pigment granula in the supporting cells of the human olfactory epithelium are also secretory, because of their deliquescence and the presence of secretion spaces.

§ 6. THE MEMBRANA LIMITANS OLFACTORIA OF VON BRUNN. Many investigators — EXNER (1877), KALLIUS (1905) and SUCHANNEK (1893) — question the existence of the membrana limitans olfactoria and consider it as an artefact, the result of agglutination of the

olfactory hairs with or without a serous fluid. Light-microscopically it is seen as a thin translucent border, occasionally provided with small rudimentary cilia (KRAUSE, KALLIUS). VAN DER STRICHT (1909) and KALLIUS (1905) found bands of some firm intercellular cement to be present only between the epithelial cells. Besides this they saw a reticular membrane on top of the supporting cells. It is mostly proved in the course of time that fierce opponents in scientific problems all are right. With the electron microscope we have found the hair-shaped projections of the supporting cells, the vesiculae olfactoriae with their cilia olfactoria, sometimes common cilia (fig. 1 and see Part II), a cuticular border bounding all surface structures, and moreover a real cuticular membrane with partitions between the epithelial cells. According to VON BRUNN the partitions surrounded only the dendrite, but in our preparations they also partly surrounded the sensory cell perikaryon and axon. We propose to call this real cuticular structure henceforth the membrana limitans olfactoria. This membrane is found in type III olfactory epithelium of the cat, situated posteriorly in the olfactory area. However, in preparations of olfactory epithelium of young humans (fig. 40) and in preparations with increased fixing time of types I and II olfactory epithelium of the cat a slender partitional network is visible just under the surface. However, these partitions are very much thinner than in type III olfactory epithelium of the cat ($> 1\ \mu$).

Earlier investigators were of the opinion that the membrane was an artifact resulting from the agglutination of the hair-shaped projections with or without secretion, or possibly a conglomeration of these projections. This theory is conclusively contradicted by the electron microscopical study of preparations made after different periods of fixation (page 39). It was not possible to us to determine whether these partitions are an intercellular product or intracellular part of the supporting cells. The granular substance they are composed of we saw located within the walls of the supporting cells:

1°. in the cuticula, which is the syncytial top of the supporting cells (fig. 17);
2°. in the branching basal part of the supporting cell.

On the other hand we did not find this substance within the olfactory cell, whereas the membranes of both the olfactory cells and the supporting cells lie outside the granular structure of the partitions (figs. 7 and 15). The partitions may be considered as an intercellular connecting element derived from the supporting cell, located mostly just at the border of the olfactory and the supporting cell. They proved to be connected with the excretory ducts of the Bowman glands (fig. 18 and drawing 3). There seems to exist a system of intercellular substance enveloping the supporting cells, the olfactory cells and the Bowman gland ducts. A possible function may be insulation and support of the sensory elements.

§ 7. THE HAIR-SHAPED STRUCTURES ON THE SURFACE. As described by BLOOM and ENGSTRÖM, (1954) from the supporting cells protrude projections which do not always end somewhat thicker than a finger-tip, as these authors suppose, but they are mostly basally wide and apically thin. Only occasionally an apparent widening at the top was

seen as described by BLOOM (fig. 16). Both on cross and on longitudinal sections they seem to be tubularly limited by a dense membrane.

According to BLOOM and ENGSTRÖM these "hairs" resemble the projections between the cilia of the epithelium of the upper respiratory tract. They assume these tiny hairs to be possibly preciliary. These projections also resemble the hair-shaped structures on top of Hensen's cells of the organ of Corti, the structures on the supporting cells of the cristae ampullares (WERSÄLL, 1956), the projections of the surface of the thyroid-cells seen by MONROE (1953), and those of the epithelial cells in the kidney, stomach and intestines which were found by other investigators. ENGSTRÖM and BLOOM suppose their function to have something to do with resorption and secretion. Some authors, inter alia MONROE call them "microvilli". He finds these microvilli to have an average length of 0.19 μ. ENGSTRÖM and BLOOM find the projections of the supporting cells to have a length of 1—2 μ, whereas in the present study a length of $\pm$ 3 μ has been found. They usually seem to be broken off, as a result of the manipulations they have been submitted to. Therefore we assume that they have a greater length than shown in the preperations and that they are not quite the same as the microvilli described by several other authors. Perhaps they yet have a secretory function (see page 43). Moreover, these projections form the skeleton between which secretion can accumulate and by which it is firmly kept together. The third possible function seems to be that of supporting the vesiculae olfactoriae and cilia olfactoria which seem to rest upon them. From this it appears that the supporting cells, by means of their tubular processes, also give support to the most peripheral part of the olfactory cell.

Bloom found the olfactory cilia of the frog and toad to be identical in structure to all kinocilia, which we could confirm with regard to the cilia olfactoria of the cat. As FAWCETT and PORTER (1954) did in the non-sensory cilia, we found a basal cross-plate in the basal body. In contrast with the findings of FAWCETT and PORTER in ordinary cilia there seems to be a structure interconnecting the basal bodies in the continuation of the basal plate. These interconnections are only seen in cross-sections. Whether they run in circles, in ovals or in a spiral cannot be ascertained. The true length of the cilia olfactoria is difficult to determine; BLOOM (1954) has found them to measure up to 40 μ, GASSER (1956) up to 100 μ. In the cat we found only a length of $\pm$ 3 μ. We have not seen a cilium divide into long tapes, as described by GASSER.

So two kinds of hairshaped structures were observed on the olfactory epithelium, similar to those described by SCHULTZE (1862) and HOPKINS (1926). BLOOM only found the olfactory cilia. SCHULTZE and HOPKINS first described in vivo the long *immobile* structures basally thick and apically thin (75—150 μ in length) which to us seem to be derived from the supporting cell. In the second place they described short *mobile* (20—50 μ) structures probably identical to the cilia olfactoria. In our preparations both types are considerably shortened, as the result of the manipulations they were subjected to. SCHULTZE and HOPKINS have noted this shortening too. The mobility of the olfactory cilia may be explained by the presence of the basal bodies from which they emanate and which probably act as a "kinocentrum".

§ 8. THE BASAL CELLS. According to GASSER (1956) the membrane of Schwann merges into the plasma membrane of the basal cells. This author is of the opinion that the bundles of axons push the plasma membrane of the basal cells back and that invaginations can be formed at such a depth that the inner walls of the invaginations and the rest of the cell membrane touch, in this way forming double membranes around the axons. This is in analogy to the findings of GEREN and SCHMITT (1954) who showed that the myelin sheath consists of a membrane encircling the axon like a spiral and arising from the plasma membrane of the Schwann cell. The basal cells will be discussed extensively in part II in connection with the regeneration process.

§ 9. THE GLANDS OF BOWMAN. The different cell-shapes described by PLANEL (1951) are also seen in the cat. As all forms are seen in the same area, the shape does not seem to depend on the amount of the subepithelial tissue and the number of olfactory bundles. Both in man and the cat the gland cells are found in various stages. Cells in the same stage are lying in groups around a duct, somewhat intermingled with other groups, yet there are various layers distinguishable in which duct cells in one special stage dominate. At the bottom of the Bowman gland, deepest in the subepithelial tissue and remotest from the olfactory epithelium are found the small indifferent cells lacking secretion granula and with only few mitochondria, possibly in a stage of development. SLOTWINSKY (1932) has found these cells by light microscopical investigation. He suggests, that the cells are either exhausted or undifferentiated ones, serving for regeneration. No mitoses are seen. This stage is mostly found in a young cat's and in a human baby's abnormal olfactory epithelium.

In normal epithelia ducts consisting of gland cells with large pigment granula not surrounded by secretion lie remotest from the olfactory epithelium. These cells are supposed to be in the presecretory stage. Somewhat more superficially are found the secretory acini provided with cells containing pigment granula and secretion spaces. Since the pigment granules are found to lie in the middle of the secretion spaces, deliquescence of the secretion may be supposed to begin at the outside of the granules. Smaller granula lie excentrically in their secretory spaces. Granular structures remain adhered to the separating membrane of the secretion cavities. They seem to be remnants of the secretion granula; they also adhere to the limiting membrane of the cavities, sometimes lacking secretion.

Apically situated granular structures around mostly empty cavities, and basal secretion cavities are found in some cells, so it may be assumed that in the beginning of the secreting period secretion is formed apically in the cell, and at the end basally (fig. 21). The secretion spaces resemble those of the thyroid gland in the active stage, but they are much smaller (fig. 21). Cells in the active secretory and in the presecretory stage are unfrequently found in pathological olfactory epithelium, they are common in full-grown epithelium storing many pigment granula both basally and subepithelially.

We could confirm the presence of intercellular canals as described by BLOOM in electron microscopical and by SLOTWINSKY in light microscopical studies (fig. 21). In the present study the tiny hairy protoplasmic projections are found both in the

intercellular canals and the gland ducts. These structures are similar to the corresponding structures of the supporting cells, though they are shorter and slenderer. They, too, resemble the projections found by other investigators on the epithelium cells of the kidney, stomach, intestines, gall-bladder, thyroid gland and on the Hensen's cells of the internal ear and the supporting cells of cristae ampullares. Their secretory function can be inferred from their tubular structure and from the fact that the secretion lies on top of them. Besides, by way of these projections the secretory product may diffuse through the gaps of the cell membrane which are covered by a thin membrane. Because the projections fill the lumen for the greater part, they retain the secretion in the same way as do the hairs of the supporting cells. Their secretory possibilities are also analogous to those of the supporting cell projections.

Closely underneath the olfactory epithelium — more profusely in abnormal olfactory epithelium — lie the gland cells of Bowman at rest. They present mostly empty cavities surrounded by tiny granular structures, adherent to the limiting membranes, which structures seem to be remnants of the pigment granula. SLOTWINSKY (1932), also, saw that the upper part of the glands of Bowman lack mucous granula. So we distinguish four stages in the gland cells of Bowman: the depôt or developmental stage, the pre-secretory stage, the secretory stage and the post-secretory or rest stage.

PLANEL (1951), in the rat and mouse, also saw the secretory products lying interstitially. In the present investigation there is found in the cat a complete system consisting of the glands of Bowman with their excretory ducts, containing protoplasmic projections, intra-epithelially surrounded either by the olfactory cells or the supporting cells. In epithelium type III, the ducts are also either or not connected with the partitions of the membrana olfactoria. From this relationship it may be inferred, that there are transitional forms between the gland cells, the basal cells, and the epithelial cells, which possibility will be discussed in part II.

PART II

ELECTRON MICROSCOPY OF PATHOLOGICAL AND REGENERATING OLFACTORY EPITHELIUM

CHAPTER V

EMBRYOLOGY OF THE REGIO OLFACTORIA

FINDLAY (1894) asserted that the sheath of the olfactory nerve fibres could not be compared with the Schwann sheath. According to him the sheath of the olfactory nerve fibres, during development, remained in the stage of an embryonic neurolemna. LAMS (1940) compared the supporting cells with the ependymocytes of the neural canal. Therefore he called them ependymic cells and on account of their glandular structure and their fibrils he attributed to them a function similar to that of neuroglia cells. The basal cells resembled oligodendrocytes which he compared to Schwann cells. Because of their possibly lipoid character the partitions around the central parts of the olfactory cells of type III olfactory epithelium can be compared with the myelin sheath around the axons in the other parts of the nervous system. As the oligodendroglia cells form the myelin around the axons in the central nervous system, the supporting cells may with their central branches form the sheath around the intra-epithelial central olfactory fibres. All this indicates the very primitive embryonic character of the olfactory epithelium. Therefore we studied the different theories about the embryology of olfactory epithelium.

In mammals the olfactory epithelium originates from an ectodermal thickening at both sides of the prosencephalon, the olfactory placode. It forms the olfactory groove, which becomes deeper and deeper, and at its bottom develops the olfactory epithelium. Various opinions about its origin have been formulated. In the first place the ectodermal origin theory of DISSE (1897), who on the inner side of the olfactory groove saw many cells in mitosis from which pearshaped elements arose identical with neuroblasts. The processes of these neuroblasts were directed towards the mesoderm. Later on an olfactory nerve was seen to arise from the olfactory groove epithelium and to proceed as far as the frontal lobe area without being connected to it.

According to His the nerve also consisted of strings of bipolar nerve cells which later on formed an "olfactory ganglion". The latter became the bulbus olfactorius. The olfactory tract was formed from this bulbus and in the second month it reached the cerebrum. DISSE (1900) demonstrated that the central processes of the neuroblasts form the olfactory nerve. He saw no ganglion, but he did see unipolar ganglion cells, resembling neuroblasts, which lay along the course of the nerve in the mesoderm with their offshoots directed centrally. There were also a few bipolar cells with offshoots going both to the brain and the epithelium. The cells lying along the nerve arose from the

epithelium and formed a sort of "Schwann" sheath. Later on the neuroblasts which remained in the epithelium developed a peripheral offshoot which became a protoplasmic process and ended in a node.

Since there was some relationship between the olfactory organ and the neuroporus anterior, the idea of a nervous origin of the olfactory epithelium was also entertained. GROTH (1938) stated that in the rabbit a thickening of the edge of the neuroporus anterior occurred, which edge was a rest of the neural plate, separated from the cerebral anlage. LAMS found that in the guinea pig the placode epithelium lay upon the future brain tissue. PLANEL (1951), in his embryological studies, could confirm the neurectodermal origin of the olfactory placode in the rabbit.

PLANEL reported two sorts of nuclei in a thirteen-day old mouse embryo, viz.: at the surface, elongated nuclei in elongated cells, present throughout the entire thickness of the neuro-epithelium and resembling ependymocytes, and deeper down eight layers of cells provided with round nuclei and both central and peripheral processes, which were neuroblasts. Two layers of mitotic cells could be seen: one superficially and one in the basal layer. In the latter lay the indifferent cells. In the mouse embryo the basal layer was present before the glands of Bowman developed. In the rabbit embryo, on the 24th day, epithelial nodes penetrated the subepithelial tissue and from them the glands of Bowman had their origin. At the same time the basal layer developed.

VAN DER STRICHT (1909) used tangential sections for an accurate study of the histogenesis of the superficial formations in the olfactory epithelium; he differentiated a first and a second stage. In the first stage he saw a layer consisting of a number of rows of nuclei surrounded by a thin zone of cytoplasm with peripheral and central processes. At the surface polygonal fields with one or two diplosomes were visible, in a superficial layer there was a sort of intercellular cement between the epithelial cells. He found a marked analogy with the tangential sections of the medullary epithelium and the brain vesicles, from which the sensory epithelium of the auditory organ and outer part of the retina arose. As already demonstrated by LAMS and PLANEL, the olfactory organ resembles an ontogenetically early stage of the auditory and optic sensory epithelia.

In the second stage VAN DER STRICHT described a differentiation of the polygonal fields into larger and smaller areas. The larger ones belonged to the future supporting cells and the smaller polygones to the future olfactory cells. However, there were also areas of undifferentiated cells, mostly multiplying cells. The sensory fields became smaller and smaller and in proportion to their smallness they became rounder. Finally, on the surface of these sensory fields very small elevations arose: the future vesiculae olfactoriae. In this stage of differentiation centrosomes multiplied by division of the pre-existing central bodies, which lay in olfactory fields in statu nascendi. The multiple central bodies moved to the periphery of the vesicle. These central bodies became diplosomes from which the cilia olfactoria sprouted. Yet a centrosome was left in the centre, both distal and proximal to the nucleus. In embryos VAN DER STRICHT still found the nuclear zone in close proximity to the surface of the epithelium, but in a further developmental stage he found it lying lower in the epithelium.

CHAPTER VI

GENERAL PATHOLOGY OF THE OLFACTORY EPITHELIUM

Many investigators of the normal olfactory mucosa experience difficulties in obtaining normal epithelium, because laboratory and other animals in captivity readily get infections of the upper respiratory tract.

In the literature there is very little to be found pertaining to the pathology of the olfactory epithelium. Both SCHULTZE (1862) and SUCHANNEK (1892) saw transitional forms in human olfactory epithelium cells, inter alia transitions into ciliated cells with pigment in their uppermost part. At various places under this ciliated epithelium ran olfactory nerve branches, but no or few olfactory cells were seen within it; there were, however, patches of non-ciliated epithelium with olfactory cells. They considered the transitional epithelium to be due to a superficial destruction resulting from catarrhal processes.

TAKATA (1929) reported that in the rabbit a severe lesion of the olfactory bulb was followed by degeneration of the entire olfactory region. Subsequently ciliated epithelium developed as seen in the respiratory portion. In case of "moderately severe" lesion the thickness of the epithelium decreased, but nevertheless it remained recognizable as olfactory epithelium. Regeneration of the olfactory epithelium he did not see. He was of opinion that the trigeminal nerve, via its ethmoid nerve branch, could be considered as the trophic nerve of the olfactory area. In a lesion of both the olfactory nerve and the trigeminal nerve degeneration of the olfactory mucosa was much more marked than when the olfactory nerve alone was severed. Ipsilateral to the lesion there was a striking widening of the Bowman gland ducts.

LE GROS CLARK and TURNER WARWICK (1946) did not see any regeneration of the olfactory epithelium in the rabbit, neither after partial nor after complete destruction of the olfactory bulb. After 24 hours already the epithelium had decreased in width and sensory cells had diminished in number. Pyknosis and karyorrhexis as well as granules of nuclear débris were seen. The peripheral projections of the olfactory cells showed distortions and varicose swellings, while the terminal swellings and olfactory hairs were disintegrated. After 48 hours the disintegration products had been taken up in large vacuoles of the supporting cells. PLANEL (1941) made the same observations. According to LE GROS CLARK and WARWICK it was questionable whether or not there were macrophages; they did not see any mitoses. After 8 weeks retrograde degeneration was complete. At that stage the epithelium was half its original thickness, and only

supporting and basal cells were left, the nuclei of the supporting cells lying at midlevel. There was atrophy of Bowman glands and contrary to TAKATA development of epithelium with cilia was not seen. In local olfactory bulb lesions, they saw the lesions spread through the entire olfactory region. There was a general thinning out of the receptors. When they repeated these experiments in 1950 and 1951 in the rabbit there appeared to be a certain degree of regional projection of the olfactory epithelium on the bulbus, as already reported in § 1 of chapter IV. LE GROS CLARK (1956) described, however, that a considerable portion of olfactory cells persist.

In normal guinea pigs PLANEL (1951) seldom found the so-called intermediate or mixed type of olfactory epithelium in the olfactory epithelium area but he found this type to predominate in castrated male animals. This intermediate type has a strongly decreased number of layers in comparison with the normal epithelium. The basal layer consists of sensory cells, above which columnar epithelium with cilia is found. Many neuroblasts were found in nests of cells at the level of the invaginations in the epithelium caused by the glands of Bowman. In the subepithelial tissue there was a cellular inflammation-like infiltration with a marked vascular congestion, as a result of which the Bowman glands were compressed and narrowed. PLANEL, as well as LAMS (1940), found an infiltration with lymphocytes, but no leucocytes. Superficially in the epithelium of castrated animals, LAMS found some sort of round cyst lined by a kind of endothelial cells filled with an unstainable substance and several intertwined threads. PLANEL did find these cysts in the neighbourhood of Bowman glands but LAMS saw no relationship between them and the excretory canals. They were neither connected with lymph vessels nor with capillaries and LAMS suggested that they were caused by a local degeneration of pre-existing tissue.

FRÜHWALD (1935) described, in rabbits, the atrophy of the olfactory epithelium and of the olfactory bulb and piriform lobe, after occlusion of the nostrils. SMITH and GRAIGIE (1937), in apparently healthy white rabbits, occasionally found destruction of the olfactory cells with atrophy of the central projections, the remaining epithelium being changed into ciliated columnar epithelium. They supposed this to be caused by chronic inflammation. PLANEL finally saw, in pathological olfactory epithelium, atrophy of both the epithelium and the subepithelial tissue. He counted three layers of cells instead of ten: the cells were chaotically distributed and the basal layer was interrupted. There was no clear segregation from the subepithelial tissue, and numerous neuroblasts were lying mixed with columnar epithelium cells. Superficially a squamous aspect was produced, all the cells together forming a syncytial covering layer. There were hypertrophic nuclei with nucleoli, probably of nervous origin. Nowhere the respiratory type of epithelium was found. The layer of basal cells was present. There were normal nerve fibres and there was neither infiltration, vasodilatation nor interstitial edema. Bowman glands had changed to narrow ducts with only a low secretory activity and there was an almost complete disappearance of the mucus at the apical pole. The surface of the olfactory epithelium was covered with débris of nuclei and shed-off cells mixed with mucus. Only far posteriorly in the nasal cavity there was normal olfactory epithelium,

and this only at one side. PLANEL, however, contrary to the findings of SMITH and GRAIGIE, found the olfactory bulb to be intact. The respiratory epithelium with its glands was also undamaged.

SUCHANNEK (1892) reported, in pathological epithelium, columnar cells with cilia and elongated cells with elongated nuclei. However, in ozaena and chronic rhinitis he found metaplastic epithelium in the olfactory region. SCHUMACHER (1925), in supposed normal olfactory epithelium, described superficially situated round nuclei in the so-called protoplasmic border. According to him these cells may be either leucocytes or olfactory cells with their nuclei lying at an atypical place.

CHAPTER VII

REGENERATION OF THE OLFACTORY EPITHELIUM

KOLMER (1927) and SUCHANNEK (1892) described a replacement of the olfactory epithelium by a non-sensory, respiratory epithelium originating from the surrounding cells. SMITH (1937) and VINNIKOV (1956) saw, in pathological epithelium, regeneration of the epithelium originating from the remaining supporting and basal cells and from the cells of the Bowman gland ducts. Neither olfactory fibres nor cells were present, only ciliary epithelium.

According to LAMS (1940) the supporting cells degenerate after some time and regeneration ensues from the replacement or basal cells. He states that, if the olfactory cells lack division potentiality, a number of them disappear for ever, after each trauma. Only the ganglion cells or pigment cells of the retina in Urodeles have such a post-embryonic proliferation potential. YOSHIHIKO NAGAHARA (1940), after cutting the olfactory nerve in the mouse, saw degeneration of the olfactory nerve fibres proceeding from the place of severance to the olfactory cells. Like LE GROS CLARK and WARWICK (1946, 1950), NAGAHARA found that the supporting cells and the basal cells did not degenerate. On the third day after severance, disappearance of the olfactory cells was seen and regeneration began with mitoses of the basal cells. According to NAGAHARA normal olfactory mucosa has only scanty mitoses confined to the basal portion. There are two kinds of epithelial cells in the regenerating epithelium: active cells with small round nuclei rich in chromatin (4.5 μ in diameter) which lie superficially, and in the basal part the so-called olfactory cells with oval, relatively large, pale nuclei (5—5.5 μ in diameter). In the regenerating olfactory cells the central projection grows out first and later on the peripheral projection. The centripetal growth of the remaining and regenerating axis cylinder progresses slowly, and finally ends again in a glomerulus of the olfactory bulb. In contrast to TAKATA (1929), NAGAHARA found that after both the nervus ethmoidalis trigemini and the olfactory nerve had been severed, there appeared a similar degeneration as when only the olfactory nerve had been sectioned. According to LE GROS CLARK the so called intra-epithelially situated trigeminus nerve endings are reticulin fibres which penetrate basally. When NAGAHARA removed the olfactory bulb of the mouse, he still saw regeneration of the olfactory epithelium and olfactory nerve fibres which came from the olfactory mucosa. VINNIKOV (1956), investigating rats, saw no regeneration of the olfactory epithelium.

PLANEL (1951), however, described that after sectioning the olfactory bulb in guinea

pigs, there was a new neuronogenesis which arose from pre-existing neuroblasts; no regeneration started from the basal layer. He saw Bowman gland cells with pycnotic nuclei degenerate and finally disappear. In the basal layer and in the columnar epithelial layer he saw many mitoses especially around the necks of Bowman glands. The cells "born" from the basal layer united around the necks of Bowman glands just under the surface of the epithelium, thus forming new glands of Bowman. He tried to substantiate this by pointing to both the simultaneous embryological appearance of the glands of Bowman and the basal cells, and the absence of both these structures in the organ of JACOBSON. SCHULTZ (1941) advocated the reverse opinion that the basal cells originate from the Bowman glands. This investigator put a one percent zinc sulphate solution into the nose of a monkey. This caused necrosis of the olfactory epithelium with peeling off from the sub-epithelial tissue, during which process the olfactory cells also disappeared. Re-epithelialization originated from the ducts and glands of Bowman and from the Schwann cells of the N. Olfactorius. Within two weeks nests of cells were seen which resembled olfactory cells, lacking the peripheral and central processes. In later stages there were abnormalities in form, orientation, grouping and distribution of the olfactory cells, together with an unusual projection of the axon and olfactory nerve bundles into the subepithelial tissue. After a few months the olfactory mucous membrane exhibited a diminished number of olfactory cells, in the original place of the destruction.

In contrast to SCHULTZ, SMITH (1938) saw no regeneration of the olfactory epithelium in the cat, the monkey and man, neither did VINNIKOV (1956) in the rat. SMITH made similar experiments in the frog in 1951. He rinsed the nasal cavity with 3 ml of a one percent zinc sulphate solution. The resulting degeneration lasted 2 days. During degeneration the underlying tissue was covered with flattened cells. Sometimes they overlapped and formed more than one layer of cells. After 3 days the continuity of these cells with the outlet ducts of the glands of Bowman was apparent. During the first fortnight proliferation was slow, but afterwards it became faster and on the 28th day the number of cells was complete again. However, the cells had little protoplasm and few round nuclei: the latter were arranged irregularly and lay close to one another. Subsequently cell differentiation started, a few olfactory vesicles already being present. Mitoses were few everywhere in the epithelium and also in the glands of Bowman. In the next 4 weeks the epithelium regained its characteristic structure and position. On the 56th day the cells were still loosely arranged and the basal layer was thicker than normal. On the 70th day the epithelium was identical to that of the control frog. Both the olfactory and the supporting cells appeared to originate from the surviving cells of the glands of Bowman.

The olfactory nerve fibres degenerated and disappeared after destruction of the olfactory nerve cells. In the 3rd week the nerve fibres began to re-appear in the olfactory nerve and they were normal on the 70th day.

SMITH also saw regeneration after puncturing the olfactory epithelium of the frog with a needle 1 mm in diameter. A blood clot formed and new connective tissue grew in it and closed the hole. The surrounding epithelium lost its characteristics and

regenerating epithelial cells and neuroblasts arose. On the 15th day there was only a single layer of cells. On the 31st day there were undifferentiated cells, while after 64 days there was a thicker basal layer which was loosely arranged. This author made a comparison with the regeneration of the retinal neuro-epithelium of the salamander in which the pigment cells of the outer edge of the retina served as a germinal layer. The retinal nerve cells retained the pigment, while the supporting cells lost it.

All the investigations discussed above are made by means of the light microscope. Up to now observations obtained by electron microscopy are lacking. It is of interest to see if this new technique will reveal facts unknown up to the present.

CHAPTER VIII

METHODS AND MATERIAL

Pathological olfactory epithelium was accidentally found in the cat and in man suffering from rhinitis. The normal structure of olfactory epithelium was absent. This is relatively common in laboratory animals and can possibly be attributed to the fact that they have a lower resistance to infection than animals living in freedom. A possible regeneration of this pathological epithelium was studied in cats V and XIV and Homines II and III.

Regeneration was also studied in a litter of four kittens born on August 17th 1955 (Cats VI up to and including IX). On that day all four were pencilled high up in the posterior part of the right nasal cavities with a one percent zinc sulphate solution. The left side was not treated as it was used as a control. In one kitten perforation of the septum occurred after this treatment. Cat VI died on August 19th '55 i.e. 2 days after treatment, probably of a chemical pneumonia. The olfactory mucous membrane was immediately removed and the olfactory epithelium appeared to be completely destroyed up to the lamina propria. Cat VII was investigated after 19 days, on Sept. 5 th '55, cat VIII after 43 days, on Sept. 29th '55. In cats VII and VIII fixation of the olfactory epithelium was accomplished in vivo by putting the cats under anesthesia and using locally drops of one percent solution of veronal acetate buffered osmium tetroxyde. Cat IX died on Oct. 13th '55 after 57 days; the olfactory epithelium of this cat could not be removed until many hours after death when post-mortem changes had already made the material unfit for use. Finally, an adult cat (cat XIII) was treated with a one percent zinc sulphate solution and after six and a half weeks the regenerated olfactory mucous membrane was removed. All tissues were fixed, imbedded and cut in the same way as described in part I.

As stated above it is very difficult to obtain human material. When surface-anaesthetics, such as cocaine, are applied the surface is damaged. The author experienced this personally when he allowed olfactory mucous membrane to be removed in this way. During inhalation anesthesia the surface and especially the vesiculae olfactoriae were also damaged. (Homo I). Goods results were obtained in a nine-month old baby (Homo II) in whom post-mortem dissection of the septum mucous membrane was effectuated. The mucous membrane was removed up to the lamina cribrosa and the entire olfactory membrane was removed. In the material removed during operations, mainly performed on patients suffering from cancer, it was possible to find olfactory epithelium. This

was the case in Homo III. This patient suffered from an occlusive rhinitis — the occlusion was caused by a carcinoma. We also tried to obtain material by submucous injection of a one percent solution of novocaine into the nasal septum; after this an attempt was made to remove olfactory epithelium with a pair of Blakesley forceps. However, the olfactory epithelium was so much damaged that it could only with difficulty be recognized under the light microscope. This concerned a man, who had suffered from a chronic sinusitis with anosmia for thirty-five years (Homo IV).

CHAPTER IX

ELECTRON MICROSCOPICAL OBSERVATIONS ON PATHOLOGICAL OLFACTORY EPITHELIUM

In cats V, XIV, Homines II, III and IV, pus is found in the nose cavities. Leucocyte and lymphocyte infiltration occur in the respiratory part. Leucocyte infiltrations are never found within olfactory epithelium when the nasal mucous membrane is inflamed; lymphocytes are present occasionally. In the olfactory region we often found respiratory cilia, when making light microscopic (fig. 1) or electron microscopic investigations (figs. 27, 28, 30). Glands of Bowman and large nerve bundles were present underneath the epithelium.

In the cats V, XIV and Homo II a zone devoid of nuclei can be found superficially (see fig. 27 and 28) while under it a few layers of moderately osmiophilic nuclei are visible. Cell membranes are sometimes absent (fig. 28). Between as well as under the moderately osmiophilic nuclei a few osmiophilic nuclei are sometimes found. The surface of the epithelium is hilly and covered with hairy structures $\pm$ 0.1—0.15 μ in diameter, while the cilia of the respiratory epithelium measure from $\pm$ 0.2—0.25 μ. They contain basal bodies with root-shaped processes, and in their centre lies a bundle of fibrils. These basal bodies also have a smaller diameter: 0.15 μ, although the length of the basal body is 0.5—0.6 μ, which is only slightly less than that of the cilia of the respiratory epithelium. Generally the length of cilia is changing, but the cilia of the olfactory epithelium are on an average shorter (3—3$\frac{1}{2}$ μ) than those of the nasal respiratory mucous membrane ($\pm$ 8 μ in length) (Figs. 29 and 30). In the present electron microscopical study the cells with cilia were also seen in groups, forming as it were islands (through the light microscope: fig. 1, and with the electron-microscope: fig. 27). Vesiculae olfactoriae are never found between these cilia. However, secretory goblet cells as found in respiratory epithelium are absent.

In Homo II in an island of ciliary epithelium and in the transitional area between this island and the one provided with tubular projections a small basal body with a rootlet appears occasionally underneath a tubular projection. Sometimes one or two small fibres originating from the basal body are visible in some of these projections (fig. 27 arrow 4). In the cell covered with small cilia the number of electron-dense structures seems to have increased. Some moderately osmiophilic structures, in which two markedly electron-dense granules, are also present (arrow 2, fig. 27). In the adjacent supporting cells either small vacuoles or longer secretion spaces can be seen superficially (fig. 27 in man; fig. 16 in cat V).

Figure 27. Human olfactory epithelium of a 9-month-old child (Homo II) suffering from a rhinitis. Magnified 4000 ×. An island of small cilia (arrow 1) is visible between the protoplasmic projections of the supporting cells. The basal bodies can clearly be seen at the bases of the cilia. The number of electron-dense structures seems to have increased in the cell bearing small cilia. Some long moderately osmiophilic structures, in which two markedly electron-dense granules, are also visible (arrow 2). A vesicula olfactoria cannot be seen. A few protoplasmic projections of the supporting cells can be seen among the small cilia. Some of the protoplasmic projections are clearly provided with a basal body exhibiting a rootlet (arrow 3). From such a basal body a tiny fibril arises in the protoplasmic projection (arrow 4). Top right an edematous cell surface (arrow 5). Some vacuoles are visible.

Figure 28. Olfactory epithelium covered with microcilia. (cat V). Magnified 2000 ×. Underneath a nucleus-free zone the oval supporting cell nuclei can be seen. The cell surface clearly shows hill-like formations. No secretion formation and no vesiculae olfactoriae are visible. In the centre a defilement.

Figure 29. Human ciliated respiratory epithelium. Magnified 10.000 ×. The diameter of the cilia is 0.2—0.25 μ, its length 8 μ. The diplosome has a diameter of 0.3 μ and a length of 0.7—0.8 μ. A small, root-shaped process is protruding from the basal body (arrow 1). It has a diameter of $\pm$ 500 Å. A cilium contains a central fibre bundle (arrow 2).

Figure 30. Microcilia of olfactory epithelium of cat XIV. Magnified 10.000 ×. The diameter of the cilia is 0.1—0.15 μ, its length is 3—3.5 μ. The diplosome has a diameter of 0.15 μ and a length of 0.5—0.6 μ. In a few places the root-shaped processes can be seen to protrude from the basal bodies (arrow 1). On cross-sections the cilia contain a central bundle of two fibrils and a peripheral bundle of nine fibrils (arrow 2). The tubular protoplasmic projections (arrow 3) are visible among the cilia.

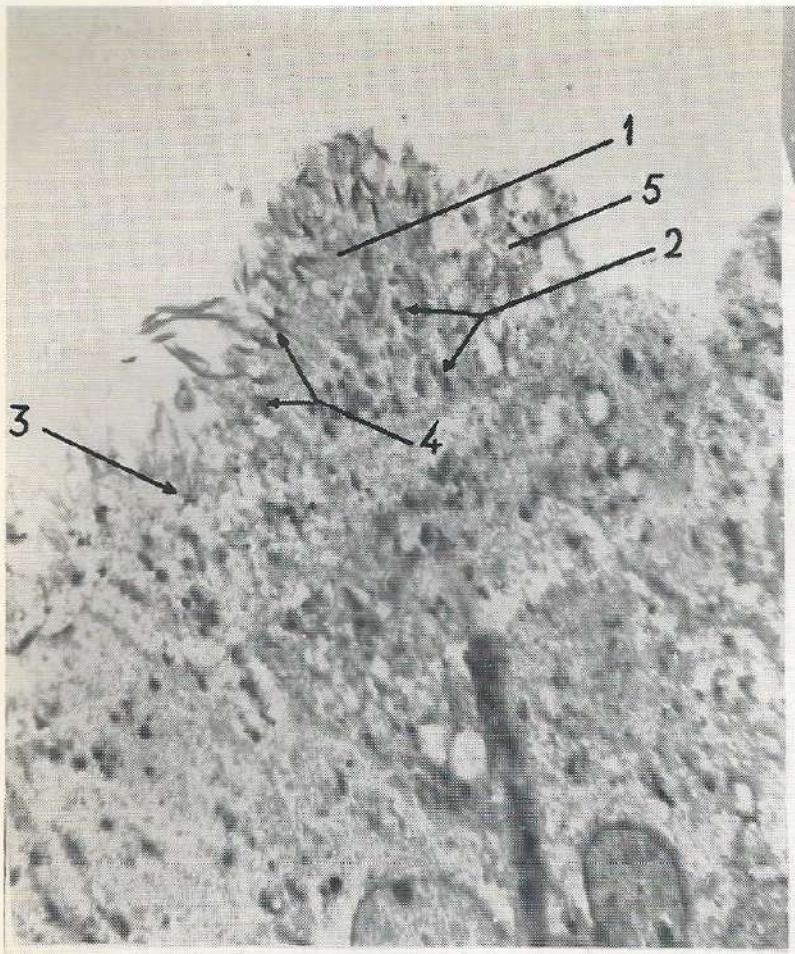


Fig. 27

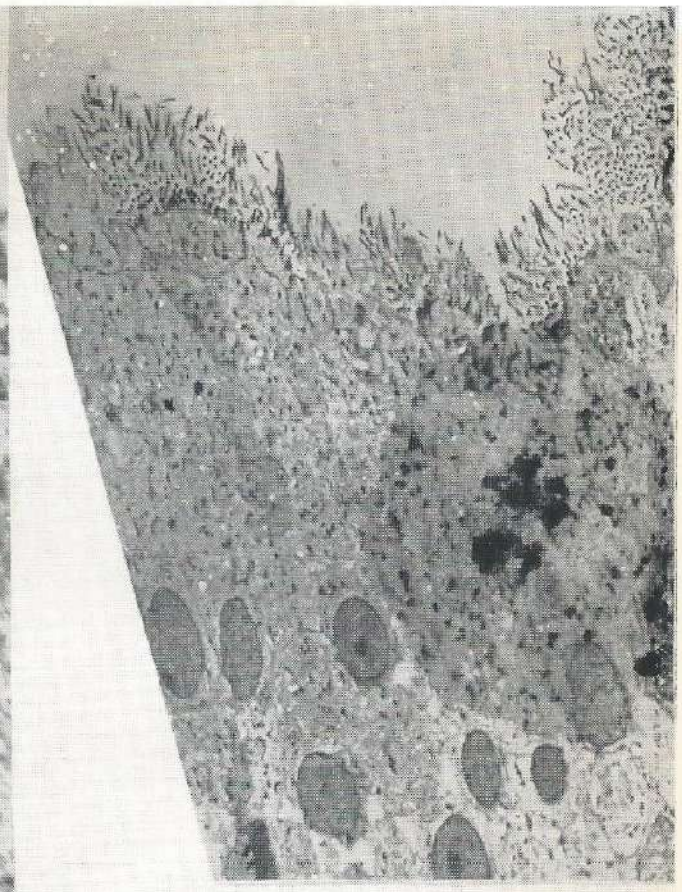


Fig. 28

Fig. 29

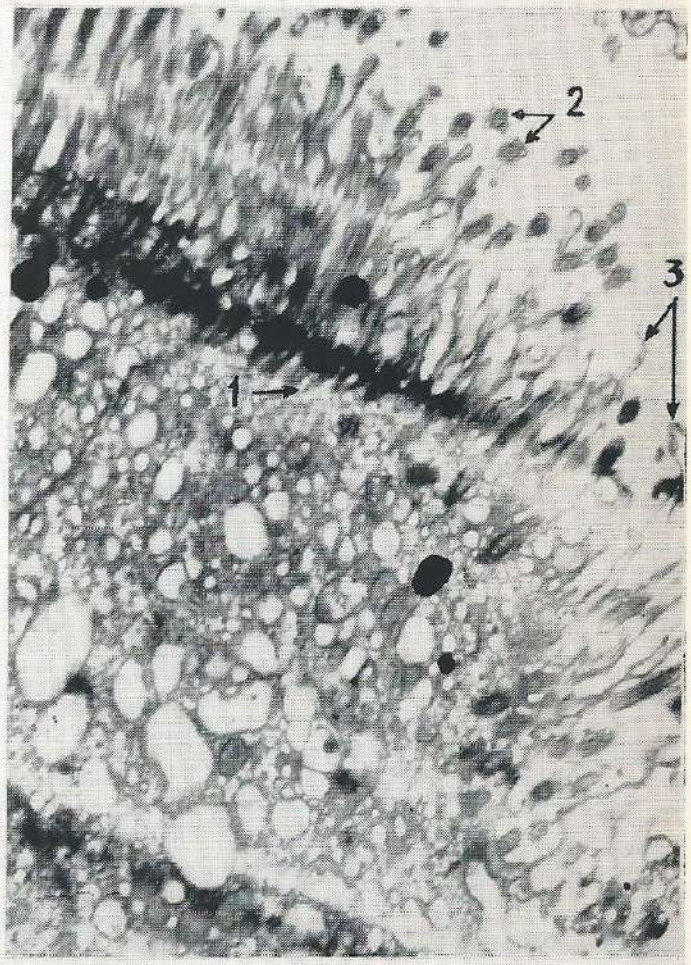


Fig. 30

In the pathological epithelium of Homo III the vesiculae olfactoriae are lacking (fig. 42), the protoplasmic projections of the supporting cells are shortened ($\pm$ 1 μ in length) and they fail to form a network. At places they are entirely absent. Very elongated cells with osmiophilic and elongated nuclei are visible. These nuclei lie more superficially than the moderately osmiophilic ones. The proximal portion of these nuclei is indented by the perikaryon of the less osmiophilic nuclei (fig. 42). These pictures are also found in cat XI. In the olfactory region of Homo IV we found large cilia and absence of olfactory cells, but in light-microscopical investigation we did see nerve bundles and glands of Bowman subepithelially.

Figure 31. Human gland of Bowman (Homo II). Magnified 4000 ×. This gland is obviously at rest and nowhere displays secretory activity, but a few granula are visible around a moderately osmiophilic nucleus (arrow 1). Centre left and at the bottom a cell with an electron-dense nucleus is seen exhibiting one protoplasmic process (arrow 2). Some other cells with more osmiophilic nuclei have an indication of a process. The other nuclei are impregnated to various degrees and contain markedly or moderately osmiophilic substance. In one protoplasmic process a Golgi-network is visible with weakly osmiophilic structures and membranes (arrow 3). The relatively thick basal membrane is characteristic for man.

Figure 32. Gland of Bowman in the developmental stage (cat VII). Magnified 1500 ×. Centrally a few cells, containing either light or dark nuclei, have a destructed cell surface. Centre right some granula have already been formed (arrow). No secretion and few small electron-dense structures are present. The lumen is surrounded by two or more layers of cells.

Figure 33. Olfactory epithelium of cat VI. Two days after destruction with a one percent solution of zinc sulphate. Magnified 2000 ×. Glands of Bowman are intact: developmental stage (arrow 1). There is a covering layer of flattened cells (arrow 2) which overlap occasionally. It can clearly be seen, that the groups of gland cells and the covering layer of cells touch each other (arrow 3). The different degree of osmiophily of all the nuclei is obvious. Arrow 4 points to a bundle of axons with a Schwann cell (arrow 5). No secretion and few electron-dense structures are present.

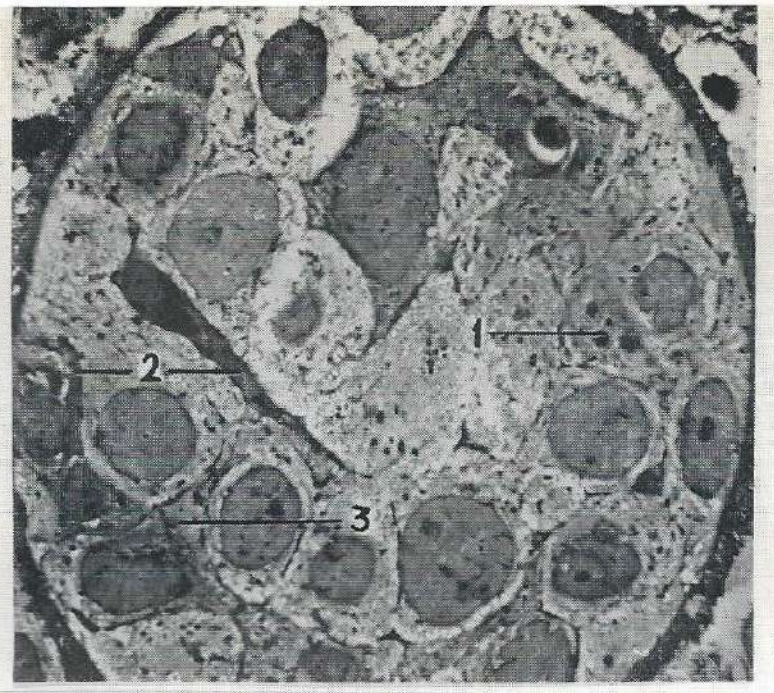


Fig. 31

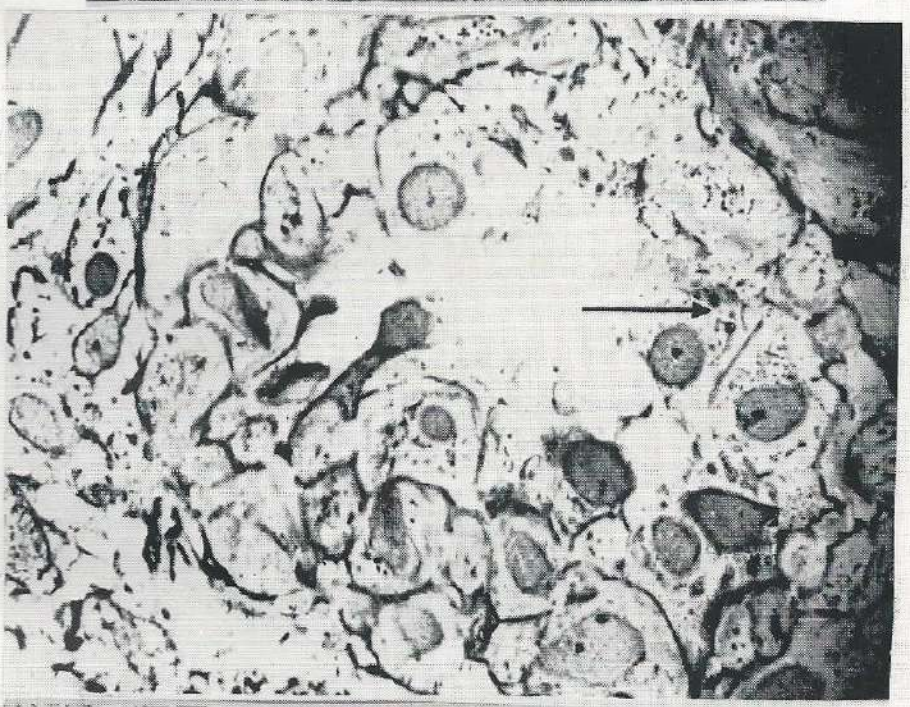


Fig. 32

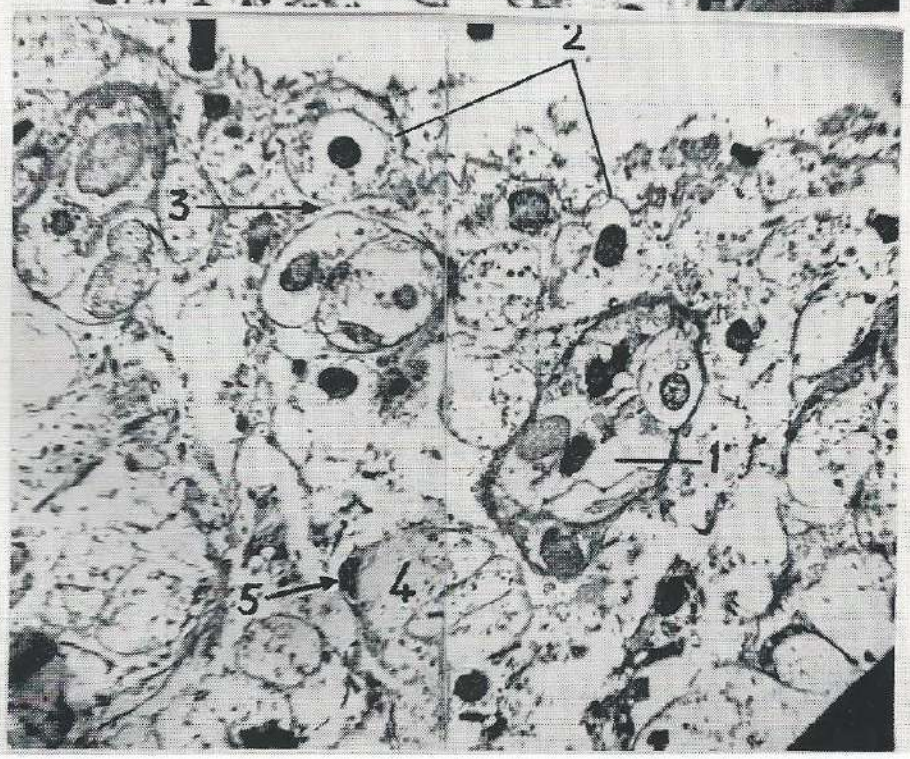


Fig. 33

CHAPTER X

ELECTRON MICROSCOPICAL OBSERVATIONS ON REGENERATION OF OLFACTORY EPITHELIUM OF THE CAT

As already reported in chapter VIII, the olfactory epithelium of four kittens from the same litter (cats VI up to and including IX) was destroyed with a one percent solution of zinc sulphate. The first one (cat VI) died after two days and total absence of olfactory epithelium was found. However, the glands of Bowman were intact as can be seen in fig. 33. The glands of Bowman are covered with a layer of flattened cells, which sometimes overlap. It is noteworthy that already some difference in degree of osmiophilia of the nuclei can be noticed in the superficial layer as well as somewhat lower, both within and outside the Bowman glands. The gland cells show neither pigment granules nor secretion rests and small electron-dense structures are few in number. These are the indifferent gland cells described in chapter IV.

The second cat (cat VII) was sacrificed after 19 days. The epithelium proved to consist solely of a syncytium containing very polymorphic nuclei, which lie in complete disorder. They were most markedly osmiophilic, although a few moderately osmiophilic nuclei were also found. In this epithelium the nuclei lay in a syncytium above the glands of Bowman. Some of these osmiophilic nuclei were already surrounded at their basal side by some protofibrils. The moderately osmiophilic nuclei were larger and rounder than the markedly osmiophilic ones.

In cat VIII's olfactory epithelium, 43 days after the destruction, the polymorphous nuclei lay in several rows in the syncytial basal layer (fig. 34). At the outside of the gland of Bowman some cells are found containing some secretion rests, surrounded by small separating membranes. The farther from the gland, the less the secretory activity of these cells. They form groups of 2—3 cells as can be seen in figure 34. In the syncytial basal layer of the epithelium just above these cell groups also lie some cells containing small secretion rests (fig. 34 arrow 3). In this layer the polymorphic nuclei lie completely unarranged. However, a few cells already show a protoplasmic central process, containing conglomerated protofibrils (fig. 34 arrow 4). More superficially the future olfactory epithelium assumes a more orderly structure and the longitudinal axis of the cells with the central processes is perpendicular to the surface. These cells have osmiophilic, elongated, oval nuclei, which vary in size. In comparison to the markedly osmiophilic nuclei the number of moderately osmiophilic nuclei is very small. The latter are less elongated, rounder and in general larger. They exhibit already

Figure 34. Cat VIII. Olfactory epithelium 43 days after the destruction Magnified ± 2000 ×. Bottom left a Bowman gland with cells containing secretion rests (arrow 1). On the right-hand side of the gland a few cells lie outside the gland. The farther from the gland, the smaller the secretion rests in these cells (arrows 2). Finally cells showing the slightest secretion activity (arrow 3) are visible in the syncytial layer of the basal cells provided with polymorphic nuclei lying in complete disorder. A few central processes containing protofibrils are visible (arrow 4). Peripherally the nuclei are more or less arranged: the longitudinal axis of the nuclei is perpendicular to the surface. There are still only a few, sometimes larger, rounder and moderately osmiophilic nuclei. Top left such a nucleus surrounded by a peripheral Golgi network can be recognised (arrow 5). Unfortunately the surface of the epithelium is damaged. Arrow 6 points to a bundle of axons. This bundle is surrounded by some cells in which secretion rests are visible.

Figure 35. Cat VIII. Regenerating olfactory epithelium. Magnified 5000 ×. The surface is not hilly and covered partly with microcilia and partly with protoplasmic projections. A central process with protofibrils (arrow) is being formed in a cell containing an osmiophilic nucleus. There is a difference in osmiophily between the nuclei.

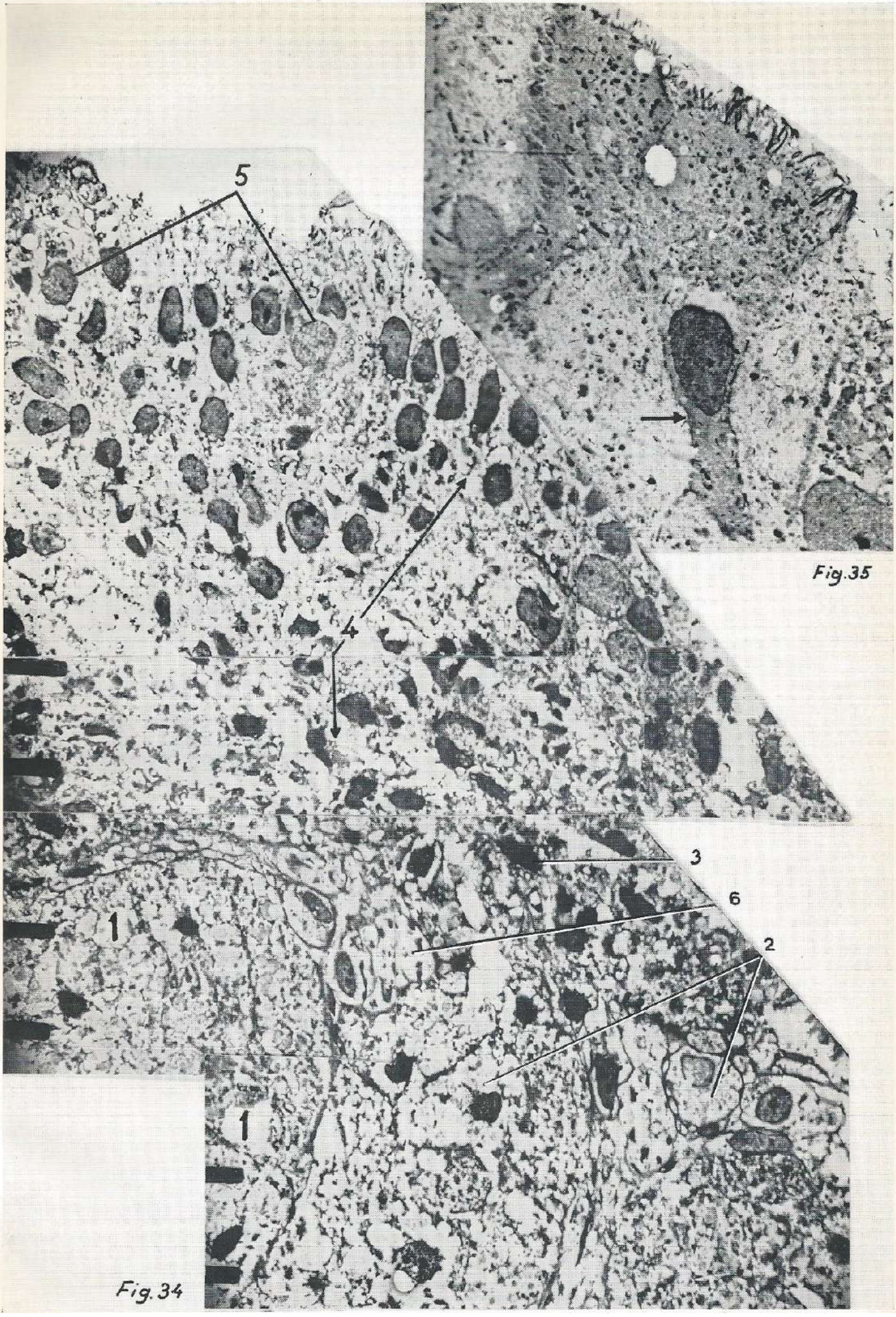


Fig. 35

Fig. 34

a scanty peripheral Golgi network in the superficial layer. Bundles of axons are subepithelially surrounded by cells containing secretion rests (fig. 34, arrow 6). Unfortunately the surface of the epithelium shown in figure 34 was partly damaged, but from comparisons with other preparations it seems probable that the surface was covered partly by microcilia and partly by tubular protoplasmic projections (fig. 35). The nuclei still lie somewhat below the surface. The central protoplasmic process of the cells with the osmiophilic nuclei is already visible, when the peripheral one is not yet formed (fig. 34 and 35). The surface is somewhat uneven.

In cat XIII about 46 days after the destruction the osmiophilic elongated nuclei are found directly under the surface (fig. 36). Several osmiophilic ring structures with radiating fibres, $\pm$ 150 mμ in diameter, arise above and in the neighbourhood of the oval nucleus (figs. 36, arrow 5 and 37). Above this nucleus and these ring structures the surface may have changed into a sort of osmiophilic cuticula (fig. 37). Next to the tubular projections above the ring structures appear osmiophilic ciliary structures. A small vesicula in statu nascendi is distinguishable, protruding on the surface and provided with basal bodies, from which several fibrils descend (fig. 36, arrow 3). After the distal olfactory cell process has been formed, the process has a conical form; within its broad base lie the nucleus and the greater part of the protoplasm (fig. 36, arrow 1) and at its top the vesicula in statu nascendi is found. Not only the vesicula, but also the most peripheral part of the olfactory cell process protrude from a deep valley. One wall of this valley is formed by the future supporting cell and the other wall by the peripheral process itself with the vesicula olfactoria. The olfactory cell nuclei are mostly oval or elongated in shape, osmiophilic and rich in chromatin.

Figure 36. Cat XIII. Olfactory epithelium 46 days after destruction. Magnified 7500 ×. Two vesiculae olfactoriae in statu nascendi are visible in their valleys. The peripheral olfactory cell process broadens basally (arrow 1). The olfactory cell nucleus which belongs to this process, is tangentially sectioned (arrow 2). Basal bodies with a single cilium olfactorium (arrow 3) can be distinguished already in the vesicula olfactoria in statu nascendi. A deep valley is visible on both sides of the left vesicle (arrow 4). So one wall is formed by the future supporting cell and the other wall by the peripheral process of the olfactory cell with its vesicula olfactoria. Some osmiophilic ring structures (150 mμ in diameter) are visible above the oval nucleus top right (arrow 5).

Figure 37. Cat XIII. Vesicula olfactoria in statu nascendi. Magnified 20.000 ×. The vesicula olfactoria i.s.n. lies in a valley. The basal bodies are already present (arrow 1). Basally in the vesicula lie electron-dense structures which each consist of an osmiophilic ring having a diameter of ± 150 mμ (arrow 2).

which possibly are analogous to the "Schwann cells" and basal cells. LAMS (1940) reported the great resemblance of Schwann cells and basal cells.

It has been suggested that in the cat after 43 days of regeneration a large number of future olfactory cell nuclei with only a few future supporting cell nuclei lie outside the gland of Bowman. Olfactory cells are possibly formed in large numbers before the supporting cells (fig. 34, 43 days after destruction). Initially future supporting cell nuclei lie in small numbers outside the gland, but they probably still retain the ability to divide.

When the artificially evoked regeneration of the olfactory epithelium of the cat is compared with the regeneration of pathological olfactory epithelium in man, it may appear that the future olfactory cells in the evoked regeneration mature rather rapidly within the epithelium itself and the basal layer (fig. 34), while in pathological epithelium maturation might already be accomplished gradually within the glands of Bowman and the basal layer (fig. 31 and 38). NAGAHARA (1940) saw mitoses in the basal cells of the olfactory epithelium of the mouse, PLANEL (1951) saw them in the cavia. The present author saw no mitoses in pathological or regenerating olfactory epithelium of man and the cat neither in the cells of Bowman glands nor in the basal cells; in none of these cases colchicine was applied. Other authors describe only very few mitoses in the gland cells of Bowman. Therefore it seems unlikely that continuous regeneration takes place throughout life.

The indifferent gland cells, lying deepest subepithelially, possibly form the stock in regenerating olfactory epithelium as SLOTWINSKY suggests in 1932. After recurrent heavy infection with destruction of the olfactory cells and subsequent regeneration, the stock of Bowman gland cells, because of their presumably lacking or decreasing potentiality for mitosis both in the cat and in man, may be exhausted, and instead of these gland cells connective tissue arises. Thus the sense of smell can diminish and even disappear. SMITH (1937) found, in autopsies of older humans, in 55 % degeneration of $^2/_3$ of the ganglion cells of the bulbus olfactorius; in 13 % these cells were absent and in 29 % the bulbus was normal. VERSTEEG (1956) found a diminishing of the sense of smell in elderly persons.

§ 4. THE REGENERATION OF THE OLFACTORY EPITHELIUM SURFACE. There appears to be a striking analogy between the embryological development of the surface of the olfactory epithelium as described by VAN DER STRICHT (1909) and its regeneration. In embryonal olfactory epithelium he reported a layer of nuclei located close to the surface. SCHUMACHER (1925) also described in fully developed epithelium superficially situated nuclei in the layer otherwise devoid of nuclei. VAN DER STRICHT described cilia on the polygones, which are the cross-sectioned epithelial cells. The osmiophilic structures which we found to consist of an electron-dense ring or an opened ring, resemble the centrioles described by HARVEN and BERNHARD (1956) in mitotic cells of vertebrates. Such a centriole presents itself as an empty cylinder 150 mμ in diameter, and 300—500 mμ in length. They state that its wall is very osmiophilic, and consists of nine tubuli, arranged parallel to the axis of the cylinder. From the centriole radiate many

fibres, which are the spindle-fibres. The centrioles are surrounded by a diffuse, osmiophilic condensation. We have not been able to differentiate the nine tubuli, because they are visible at a 60,000 × magnification only. In our preparations their diameter is sometimes a little larger, possibly owing to exaggerated osmiophilic condensation.

In normal olfactory epithelium the olfactory cell nuclei lie under the supporting cell nuclei. This may be due to one of two causes: either the olfactory cell nuclei migrate in a central direction, or the supporting cells with their nuclei grow out in a distal direction while the olfactory cells grow less fast. In this study it is suggested that both suppositions are true. After the central process and the peripheral process with the centriole-like structures and cilia of the vesicula olfactoria i.s.n. have been formed, the olfactory cell nuclei possibly migrate from the superficial hilly elevations to the deeper layers. This migration of the olfactory cell nucleus taking with it the greater part of the protoplasm to a deeper layer of the olfactory epithelium, may cause the protoplasmic process near the surface to become progressively narrower (fig. 36). This is completely in analogy with the findings of VAN DER STRICHT, who saw the future olfactory fields become progressively smaller and rounder during their embryological development. In tangential sections (fig. 40) of the pathological olfactory epithelium of a nine-month-old child the hexagonal supporting cell and round olfactory cell fields are distinguishable.

Because the possible migration of the future olfactory cell nucleus may occur relatively rapidly with respect to the increase of protoplasm and the growth of the peripheral process, the vesicula olfactoria i.s.n. is pulled down in a deep valley (fig. 36). The peripheral process of the olfactory cell is conical yet, but owing to further migration it becomes cylindrical. The vesicula olfactoria i.s.n. may be pushed toward the level of the supporting cell surface due to the growth in length of the peripheral process (fig. 36). In this way a deep valley is formed not only bordered by a future supporting cell, but also by the peripheral process of the olfactory cell and its vesicula i.s.n. (fig. 36). After a large number of future olfactory cells have been formed, the glands of Bowman may produce more future supporting cells; mitotic proliferation of the supporting cells in the basal layer is also possible. After this the supporting cell nuclei pass to the superficial layer and finally to the syncytial layer in order to form the supporting cells which surround the olfactory cells. This movement, which is opposite to that of the olfactory cells, may cause the cylindrical shape of the peripheral processes of the olfactory cells.

From the regeneration of the cat's olfactory epithelium, as described and discussed in this chapter, it seems that type II olfactory epithelium described in part I, is in a stage of regeneration. In this type the vesicles are found in the valleys between the supporting cell elevations. Superficially there is a syncytial layer with mostly olfactory cell nuclei. In comparison with type I olfactory epithelium, the olfactory cell nuclei lie higher, as described in § 2 of chapter III, part I. In completely developed olfactory epithelium the olfactory cell nuclei lie under the supporting cell nuclei. NAGAHARA also in regeneration described two types of nuclei, whose diameters and density of chomatin structures were analogous to those of the nuclei described by us in type II.

Contrary to us, he supposed the deeply situated nuclei to be olfactory cell nuclei, and the superficially situated nuclei to be supporting cell nuclei. The probability of the shifting of olfactory cell nuclei to the deeper layers and of the supporting cell nuclei into the periphery is demonstrated by the indentation of the olfactory cell nucleus, caused by the supporting cell body with a Golgi network around its nucleus. This can be seen in different places in figure 4, (arrow 4) which also shows that the central processes of the olfactory cells are already fully developed, whereas in the peripheral ones a distinct cell membrane is still lacking. In the basal layer are seen, besides a large number of future supporting cells with pigment granula, some polymorphic osmiophilic nuclei of replacement cells. The latter correspond to the polymorphic osmiophilic nuclei of the cells that possibly are future olfactory cells; these are seen during artificially induced regeneration in the basal layer. The superficial mitochondria-free layer in type II corresponds to the syncytial layer of the membrana limitans of type III olfactory epithelium; perhaps this membrane originates from that layer.

We may conclude, that there are only two types of olfactory epithelium instead of three: one with and the other without the membrana limitans olfactoria of VON BRUNN. All other forms are either pathological or regenerative ones.

The surface structures of pathological human olfactory epithelium (Homo III) strongly resemble the artificially evoked regenerating epithelium of the cat. The osmiophilic granula with a ring structure resemble the centriole-like structures of the cat. The opened rings are also described by HARVEN and BERNHARD (1956). The elongated osmiophilic nuclei, whose bases are indented (fig. 42) are also seen in the cat's olfactory epithelium (fig. 4). PLANEL (1951) also described superficially situated elongated cells with elongated nuclei in a thirteen-days-old mouse. SUCHANNEK (1892) reported them in human pathological olfactory epithelium. In § 2 of this chapter it is supposed that these elongated cells containing osmiophilic nuclei and provided with central processes are the olfactory cells and that the elongated nuclei are indented during their shifting between the supporting cells. Electron microscopy has shown that the vesiculae olfactoriae are destroyed and that the tubular projections are shortened. The olfactory cell nuclei, after their migration to the upper part of the epithelium, possibly organize the surface structures and induce their growth. The olfactory or supporting cells need not wholly to be destroyed by the infection.

CHAPTER XIII

SUMMARY AND GENERAL DISCUSSION

The many unsolved problems concerning the normal and pathological olfactory epithelium induced us to collect new data by means of the electron microscope. Our attention was especially focused on the olfactory vesicles, the hairlike structures upon the olfactory epithelium, the membrana limitans olfactoria of VON BRUNN, the pigment and the epithelial regeneration.

Our investigations with the electron microscope revealed different types of olfactory epithelium in the cat, especially with respect to their superficial structures and nuclear arrangement.

In the commonly known and described type, here called type I, the vesiculae olfactoriae lie above the cell surface. The protoplasmic layer, devoid of nuclei, is bordered below by a layer of supporting cell nuclei, which again is bordered by the darkly stained osmiophilic olfactory cell nuclei. Basal cells are practically absent in the fully developed epithelium.

In type II the vesiculae olfactoriae are found in the depths bordered by the walls of the supporting cells. Here the uppermost row of nuclei are the olfactory cell nuclei, under which the supporting cell nuclei lie, in contrast to type I. Under the row of supporting cell nuclei an enormous number of basal cells is visible, which leads one to surmise a possible regenerative function. In part II of the present paper the conclusion is drawn that type II is a regenerative stage of the olfactory epithelium.

In type III the existence of the membrana limitans of VON BRUNN is confirmed. In this epithelium is found a large network of partitions between the supporting and the olfactory cells, which partitions are absent in the other types. The olfactory cells and the excretion ducts of the Bowman glands are for a large part surrounded by these partitions. Directly below the surface the partitions fuse to form a syncytial cuticula consisting of the peripheral ends of the supporting cells. The cuticula may be either thick or thin and may even be completely missing in several places. It is perforated by the peripheral processes of the olfactory cells. On the surface of the cuticula there may or may not be club-shaped or cauliflower-like swellings from which extend tubular protoplasmic projections. These projections are also found protruding from the tops of the supporting cells. The vesicles lie between these swellings. Both the partitions and the membrana are composed of a dense, apparently amorphous, osmiophilic substance within which can be seen darker granules; these structures are supposed to be an intercellular or an intracellular product of the supporting cells.

Type III was found in the posterior portion of the nasal cavity of three cats. The topographical projection of this area upon the posterior part of the bulbus, demonstrated by LE GROS CLARK and WARWICK, and the greater number of action potentials in this part of the bulbus generated by smelling meat, fish or volatile oils (ADRIAN 1956) make it probable that this type is exceptionally sensitive to these substances.

The peripheral part of the olfactory neuron contains many filiform opaque structures, probably consisting of conglomerations of protofibrils, which, however, are absent in the most peripheral portion, directly underneath as well as within the vesiculae olfactoriae. There are no holes, but small vacuoles in the vesiculae olfactoriae. From the vesicle sprout at least 36—40 olfactory hairs, protruding from a basal body or diplosome. The basal cross-plates of these basal bodies are interconnected by opaque linear structures. The cilia run parallel to the surface, at least in the present preparations. When the vesicula lies in a depression, the olfactory cilia first rise perpendicularly and after emerging above the surface they run parallel to it. They rest upon the tubular projections of the supporting cells. The cilia olfactoria have the same structure as the kino-cilia as found in the cilia of the entire flora and fauna: an axial bundle of fibrils consisting of an external layer of nine paired fibrils and two fibrils in the centre; the whole is enveloped by a sheath of a granular structure, which merges into the covering cuticular protoplasm of the vesicle. This fibril bundle is also found in the basal body; it passes through the basal cross-plate and proceeds through the vesicle, where larger bundles of fibrils are formed ending between or in the filiform structures.

On cross-section the supporting cell is hexagonal in shape, the olfactory cell dendrites indenting its sides. In agreement with GASSER we found in the supporting cells both the circularly and the longitudinally arranged structures of the endoplasmatic reticulum. Superficially in the supporting cell are seen secretory products. We suggest that the spaces filled with secretion empty either via small gaps of the cuticular cell membrane or via the tubular protoplasmic projections. The supporting cell branches out downwards into offshoots of various shapes. These offshoots are often filled with pigment granula and a dense mass of granulated osmiophilic structures, connected with the partitions of the membrana olfactoria. Very little pigment granules are found in olfactory epithelium of young cats and very young humans and in pathological and regenerating olfactory epithelium. In the fully developed olfactory epithelium of the cat the pigment is generally found basally in rows within the branches of the supporting cells and subepithelially in the glands of Bowman. In man the granules are found throughout the supporting cell and the Bowman gland cell. In all these cells the granula can decrease in size by deliquescing into secretion.

In accordance with in vivo preparations of SCHULTZE and HOPKINS, with the electron microscope we found two types of hair-like structures protruding from the epithelium, both in man and in the cat. These investigators, however, recorded a greater length of the hairs in vivo. By the artificial manipulations the "hairs" may either have been broken off, dissolved or bent; therefore their real lengths could not be established in the preparations. The tubular protoplasmic projections protruding from the top of the

supporting cells, wide at the base and gradually tapering towards the periphery, usually form a plexus. They are not identical with the microvilli. The secretion is retained in this network, which, moreover, forms a skeleton upon which the vesicles and cilia olfactoria rest.

Within a basal cell are found groups of axons, each group surrounded by membranes, which according to GASSER seem to be formed by very deep invaginations of the protoplasmic cell membrane. Subepithelially this protoplasmic membrane gradually merges into the cell membrane of the Schwann cells, which is also in accordance with our findings.

Both in adult cats and in man we have found groups of Bowman gland cells in different stages of secretion, around the ducts. These groups are located in not very strictly arranged layers. The indifferent cells in the so-called depôt stage lie deepest. Such cells, which contain practically no granules and few mitochondria, are especially found in young cats and babies; they are most distinct in pathological and regenerating olfactory epithelium. Above and for a smaller part among them lie the presecretory and actively secretory gland cells, especially in fully developed olfactory epithelium. The presecretory gland cells are full of secretory granules. In the secretory gland cells the granules are converted into secretion by deliquescence beginning at their periphery. The surface of the gland cells, the intercellular canals, and the gland ducts are covered with small hair-like tubular projections, shorter and smaller in diameter than those extending from the supporting cells. The secretion layer, which lies on top of these structures, suggests a possible function of these projections with respect to the secretion. The formation of secretion seems to begin in the apical part of the cell and to proceed basally.

Still higher cells of Bowman glands are seen, entirely in the rest stage after secretion. The small holes in these cells, which mostly are devoid of secretion, are surrounded by pinpoint-like tiny granula, which seem to be the pigment granula remnants. Their nuclei, in contrast to the cell nuclei in the other stages, are either more or less opaque according to their degree of osmiophilia. Cell groups in this stage sometimes extend to and invaginate into the epithelium. Intra-epithelially above the basal layer the gland duct is no longer surrounded by gland cells, but by olfactory or supporting cells which in type III epithelium are connected with the network of partitions originating from the membrana olfactoria. It appears that the gland cells form a unit with the olfactory and the supporting cells. This makes the possible transformation of gland cells via basal cells into olfactory and supporting cells more plausible.

The occurrence of both increased sensitivity and resistance against exhaustion in olfactory epithelium with a large amount of pigment, as found by ADRIAN (1956), and of anosmia or hyposmia in albinoes as described by HUTCHINSON (1852) and OGLE (1870), indicates the great importance of pigment in the process of smelling. It also appears that the pigment granules in the supporting cells and gland cells can deliquesce into secretion. In the glands of Bowman they are actively secretory but in the supporting cells and basal cells they are mostly at rest. A large quantity of pigment in a certain part of the olfactory epithelium therefore indicates the possibility of more secretion

being formed than in other parts. From these observations it seems that probably the secretion of the glands of Bowman is necessary for smell in cats and in man.

Among the different kinds of pathological changes of the olfactory epithelium, we studied especially the results of chemical damage. Moreover we encountered infectious lesions in the greater part of our material. Descriptions of damaged olfactory epithelium are very scarce. In literature a transitional form from normal to ciliated epithelium is reported. In high power magnification we have observed cilia to be smaller in length and diameter than those of the respiratory epithelium. These cilia we call microcilia. The vesiculae olfactoriae are absent and the tubular projections of the supporting cells are shortened. The microcilia may arise from the preciliary tubular protoplasmic projections of the supporting cell. Under such a projection appears a dipolosome, from which a fibril bundle probably grows into the core of this tubulus, as a result of which this protoplasmic projection may become a sheath to the fibril bundle of a microcilium. In chronic infections these microcilia may increase in size and become real cilia; in this way the area covered with cilia may resemble the epithelium of respiratory type. However, subepithelially the glands of Bowman and the nerve bundles may persist for years. The microcilia possibly have a defensive function during infection of the nose.

The basal cells are often called replacement cells. According to several authors it is impossible to differentiate these immature cells by nervous tissue staining methods, as we also experienced. NAGAHARA (in mouse, 1940) and SCHULTZ (in monkeys 1941) described that in regenerating olfactory cells the central projection grows out first. After 43 days of artificially evoked regeneration in cat's olfactory epithelium we find a syncytial layer of cells containing elongated osmiophilic nuclei and, basally, central processes containing protofibrils. Some cells contain round moderately osmiophilic nuclei and secretion rests. After 46 days of regeneration future olfactory cells with vesiculae olfactoriae in statu nascendi are visible. These cells contain osmiophilic nuclei and are provided with central processes. In normal olfactory epithelium a different degree of osmiophilia of the olfactory cells and supporting cell nuclei also exists. It seems likely that the basal cells containing osmiophilic nuclei and central processes are the future olfactory cells and that the cells containing secretion rests and moderately osmiophilic nuclei are the future supporting cells. The same pictures are found in human olfactory epithelium.

43 days after artificially evoked regeneration in cat's olfactory epithelium, cells with secretion rests are visible in the syncytial layer. These rests decrease in quantity in the cells further away from the gland duct neck. The indifferent gland cells lie mostly in the deepest layers of the subepithelial tissue and the secretion develops gradually in the gland cells lying more upwards. So these basal cells are probably resting gland cells and may originate from the gland duct cells containing more secretion. Since the subepithelial gland ducts which have no membrana propria invaginate the epithelium,

which neither has such a membrana and since the intra-epithelial excretion ducts are surrounded by olfactory and supporting cells, it is conceivable that the cells of Bowman glands pass from below into the basal layer. Embryologically, the gland and basal cells originate simultaneously from the neurectodermal olfactory epithelium.

Because of the probable shifting of the resting gland cells of Bowman to the layer of basal cells, it is presumed that the gland cells containing an osmiophilic nucleus and sometimes provided with an osmiophilic process are the precursors of the olfactory cells. The gland cells containing moderately osmiophilic nuclei, small pigment granula and secretion rests are presumably the precursors of the supporting cells. All this is analogous to the different degree of osmiophilia of the supporting and the olfactory cell nuclei in normal olfactory epithelium. It may seem strange, that sensory cells should originate from gland cells, but the embryonic character of normal and regenerating olfactory epithelium makes it acceptable. BERGER (1926) described, in the esthesio-neuro-epitheliome rosettes of cells resembling supporting or bipolar olfactory cells, lying around a duct.

Mitoses of the immature supporting cells are supposed to be present in the layer of the basal cells. The future olfactory cells are so highly differentiated in the basal layer because of the presence of protofibrils in their central processes that they can no more divide. In repeated regeneration, after recurrent heavy infections with destruction of the olfactory cells, the stock of Bowman gland cells will be exhausted because of their presumably lacking or decreasing potentiality to mitosis; therefore the regio olfactoria will decrease in extent. This may be the cause of the decrease of the sense of smell in older persons as found by VERSTEEG (1956). SMITH (1937) found a partial or total loss of nerve fibres in the bulbus olfactorius of older persons, which points to the same fact.

We find a marked analogy between embryological development of the olfactory epithelium and regeneration, especially of the superficial structures. Embryologically, the central process of the olfactory neuroblast also appears first and only later does the peripheral process with the vesicle develop. VAN DER STRICHT already, in cross-sections of olfactory epithelium in embryological development, described large and small polygonal olfactory areas. The sensory cells arise from the small polygones, which become smaller and rounder. Centrosomes are found in the olfactory fields in statu nascendi. They subsequently divide in centrioles and move to the periphery, thus becoming diplosomes from which the cilia olfactoria originate. We saw the same process during regeneration of the surface of olfactory epithelium after an infection of the nose in the cat and after experimentally evoked regeneration. Originally, during embryological development and regeneration, the nuclei lie directly under the surface. In this manner the osmiophilic cell nuclei, together with the centriole-like structures, seem to induce the surface to form a vesicula olfactoria. After this the osmiophilic olfactory cell nuclei, together with the greater part of their protoplasm, seem to move very rapidly from their hilly elevations to the deeper layers: the peripheral processes near the surface become smaller and narrower and finally the vesiculae olfactoriae in statu nascendi lie deep in the recesses

between the adjoining future supporting cells. Later the peripheral processes may grow slowly and the olfactory vesicles may be moved towards the level of the future supporting cell surface. The supporting cell nuclei seem to proceed towards the uppermost layer and together with their perikaryon and Golgi network they indent the olfactory cell nuclei. Thus there possibly is an oppositely directed migration of olfactory and supporting cell nuclei in the syncytium.

The olfactory epithelium of the cat type II, with its vesicles in the valleys and a syncytium of the upper layers, seems to be in a stage of regeneration. So in the cat there have been found two types of normal olfactory epithelium: one with and one without the membrana olfactoria of VON BRUNN. All other forms described probably are pathological and regenerative forms of the olfactory epithelium.

In pathological olfactory epithelium of an adult man it appears that the olfactory and supporting cells need not to be destroyed by an infection. The surface structures are only damaged: the vesiculae olfactoriae disappear and the tubular projections are shortened. Underneath the surface many centriole-like structures are visible. The first row of nuclei under the surface are osmiophilic, elongated nuclei, which at their bases are indented by or lie between the supporting cells with the oval nuclei. These osmiophilic nuclei seem to migrate to the superficial layer, where they possibly induce the growth of the protruding structures.

We are well aware that the present study is only a modest contribution to the literature concerning the pathology of the olfactory organ. May our work induce others to study this subject and fill the existing lacuna.

CONCLUSIONS:

1. In the cat there are two types of olfactory epithelium: one with and one without the membrana limitans olfactoria of VON BRUNN. The former seems to occur in the posterior part of the nose and it probably is sensitive to the odour of volatile oils, fish and meat. Apart from these two all other forms described are most probably pathological or regenerative forms of the olfactory epithelium.
2. The partitions of the membrana limitans olfactoria, largely surround the olfactory cells, the supporting cells, and the excretory ducts of the glands of Bowman.
3. From the vesicula olfactoria being the peripheral extremity of the olfactory cell, protrude about 36—40 cilia olfactoria. The latter have the same structure as the cilia of the respiratory epithelium, except that the axial fibril bundle does not end in the basal body, but passes through it and through the vesicula olfactoria into the intra-epithelial part of the dendrite of the olfactory cell. The basal cross-plates are interconnected by opaque linear structures.
4. In active and completely developed olfactory epithelium, many secretory pigment granules are found in the supporting cells and in the gland cells of Bowman. The secretion of the Bowman glands may be important for smell. The gland cells of

Bowman are found in different stages of secretion: the depôt, the presecretory, the secretory and the resting stage.

5. From the surface of the supporting cells protrude tubular protoplasmic projections, which projections support the vesiculae olfactoriae with their cilia. Analogous smaller tubular projections protrude from the gland cells and in the gland ducts. These possibly have a function with respect to secretion. They are not identical with the microvilli. Moreover, so-called microcilia, which may arise from the tubular protoplasmic projections, are found in pathological and in regenerating epithelium.
6. It is suggested that the basal or replacement cells, which are the future olfactory and supporting cells, arise from the gland cells of Bowman. In repeated regeneration after repeated heavy infections, the stock of Bowman glands may be exhausted because of the presumable absence or decrease of mitotic proliferation. In this way the regio olfactoria will diminish in size and consequently the sense of smell may decrease.
7. Experimentally caused regeneration of all the superficial structures, viz. of the vesiculae olfactoriae and the tubular projections of the supporting cells, shows the same features as embryological development. Directly underneath the surface develops a syncytium with centriole-like structures; the central process of the olfactory cell develops first.
8. During regeneration of the cat's olfactory epithelium indentation of the olfactory cell nuclei may be caused by the growing out of the supporting cells in peripheral direction, and by the possible migration of the olfactory cells from the superficial to the middle layers.
9. In regeneration of the surface of human olfactory epithelium after a superficial infectious damage, there seems to exist a migration of the olfactory cell nuclei to the superficial layer, where they possibly induce the growth of the protruding structures with the help of centriole-like structures.

SCHLUSSFOLGERUNGEN:

1. Die Katze besitzt zwei Arten Riechepithelien, eine ohne und eine mit der Von Brunnschen Membrana limitans olfactoria. Letztere scheint sich hinten in der Nase zu befinden und ist wahrscheinlich empfindlich für den Duft ätherischen Ölen, Fleisches und Fisches. Ausser diesen zwei Arten sind alle anderen beschriebenen wahrscheinlich pathologische oder regenerierende Formen von Riechepithelien.
2. Die Zwischenwände der Membrana limitans olfactoria, umgeben grösstenteils die Stützzellen, die Riechzellen und Ausführungsgänge der Bowmanschen Drüsen.
3. Die Vesicula olfactoria als peripheres Ende der Riechzelle ist mit zirka 36—40 Cilia olfactoria bedeckt. Diese haben denselben Bau wie die respiratorischen Cilien, jedoch endet das axiale Fibrillenbündel nicht in den Basalkörper hinein, sondern geht durch ihn hindurch und durch die Vesicula olfactoria nach dem intra-epithelialen Teil des Dendriten der Riechzelle. Die basalen Querplatten sind durch dunkele linienförmige Strukturen miteinander verbunden.

4. Bei aktiven und vollständig entwickelten Riechepithelien findet man viel sekretorische Pigmentgranula in den Stützzellen und Bowmanschen Drüsen. Das Sekret der Bowmanschen Drüsen könnte wesentlich für den Geruchssinn sein. Die Bowmanschen Drüsenzellen finden sich in verschiedenen Sekretstadien: dem Depot, dem präsekretorischen, dem sekretorischen und dem Ruhestadium.
5. An der Oberfläche finden sich tubuläre protoplasmatische Fortsätze der Stützzellen; diese Fortsätze stützen die Vesiculae olfactoriae mit ihren Cilien. Analoge kleinere tubuläre Fortsätze werden auf den Drüsenzellen und in den Drüsengängen gefunden. Diese haben möglicherweise eine mit der Sekretion zusammenhängende Funktion. Sie sind nicht mit den Microvilli identisch. Ferner finden sich bei pathologischen und regenerierenden Riechepithelien sogenannte Mikrocilien, die möglicherweise aus den tubulären protoplasmatischen Ausläufern entstehen.
6. Man nimmt an, dass aus den Bowmanschen Drüsenzellen die basalen oder Ersatzzellen entstehen, die zu Riech- und Stützzellen werden. Bei wiederholter Regeneration nach wiederholter heftiger Infektion wird durch eventuelles Fehlen oder Abnahme von Mitosen in den Bowmanschen Drüsenzellen das Depot dieser Drüsen verbraucht. Dadurch kann die Regio olfactoria verkleinert werden und der Geruchssinn abnehmen.
7. Die künstlich ausgelöste Regeneration der Oberflächenstrukturen, u.a. der Vesiculae olfactoriae und der tubulären Fortsätze der Stützzellen, erfolgt in derselben Weise wie bei der embryologischen Entwicklung. Es entsteht ein Syncytium dicht unter der Oberfläche mit centriolförmigen Strukturen. Auch wächst erst der zentrale Fortsatz der Riechzelle aus.
8. Man findet bei der Regeneration der Riechepithelien der Katze eine Einbeulung der Riechkerne möglicherweise durch ein Auswachsen der Stützzellen nach der Peripherie und durch eventuelle Wanderung der Riechzellen von den Oberflächen nach den Mittellagen zu.
9. Bei Regeneration der Riechepithelien-oberfläche des Menschen nach einer oberflächlichen Beschädigung durch Infektion scheint eine Wanderung der Riechzellenkerne nach der Oberflächen-schicht zu bestehen, um möglicherweise mittels centriolförmiger Bildungen das Wachstum hervorstechender Strukturen herbeizuführen.

CONCLUSIONS:

1. Chez les chats on trouve deux types d'epithelium olfactif: l'un pourvu de la membrana limitans olfactoria de VON BRUNN, l'autre pas. Il paraît que le premier type se trouve dans la partie postérieure du nez et est sensible aux odeurs des huiles volatiles, de la viande et du poisson. Outre ces deux formes d'epithelium olfactif toutes les autres formes décrites sont probablement des formes d'epithelium olfactif pathologique ou qui se régénère.
2. Les cloisons de la membrana limitans olfactoria entourent en grande partie les cellules de soutien, les cellules olfactives et les canaux excrétoires des glandes de Bowman.

3. De la vesicula olfactoria, qui est l'extrémité périphérique de la cellule olfactive, naissent environ trente-six à quarante cilia olfactoria. Ils ont la même structure que les cilia respiratoires, mais le faisceau axial des fibrilles n'y finit pas dans le corps basal, mais traverse celui-ci ainsi que la vesicula olfactoria elle-même dans la direction de la partie intra-épithéliale du dendrite de la cellule olfactive. Les lames basales transversales sont liées entre elles par des structures linéaires opaques.
4. Quand l'epithelium olfactif est actif et complètement développé, on trouve beaucoup de granules sécrétoires pigmentés dans les cellules de soutien et dans les cellules glandulaires de Bowman. Les secreta des glandes de Bowman pourraient être importants pour l'odorat. On trouve les cellules glandulaires de Bowman dans plusieurs stades de la sécrétion: dans le dépôt, dans le stade présécrétoire, le stade sécrétoire et dans celui du repos.
5. Sur la surface on trouve des proéminences tubulaires du protoplasma des cellules de soutien. Ces proéminences soutiennent les vesiculae olfactoriae et leur cilia. Sur les cellules glandulaires et dans les canaux excrétoires des glandes on trouve des proéminences tubulaires analogues, mais plus petites. Il est possible qu'elles aient une fonction en rapport avec la sécrétion. Elles ne sont pas identiques aux microvilli. En outre on trouve dans l'épithelium olfactif pathologique et dans l'epithelium olfactif qui se régénère des cilia, appelés microcilia, qui peut-être proviennent des proéminences de protoplasma.
6. On suppose que les cellules basales ou de remplacement, qui seront les cellules olfactives et de soutien, proviennent des cellules glandulaires de Bowman. Une régénération répétée, causée par des infections violentes et répétées, épuisera le dépôt des cellules glandulaires de Bowman par l'absence ou la diminution éventuelle des mitoses dans ces cellules. Par là il se peut que la regio olfactoria soit réduite et que le sens olfactif soit affaibli.
7. La régénération, causée artificiellement, des structures superficielles, entre autres des vesiculae olfactoriae et des proéminences tubulaires des cellules de soutien, se passe de la même façon que dans le développement embryologique. Un syncytium à structures comparables à des centrioles se forme juste sous la surface. Et le prolongement central de la cellule olfactive s'y produit également le premier.
8. On trouve chez les chats lors de la régénération de l'epithelium olfactif une invagination des noyaux des cellules olfactives, causée peut-être par un accroissement des cellules de soutien dans la direction de la périphérie et par une migration éventuelle des cellules olfactives partant des couches superficielles vers les couches moyennes.
9. Lors de régénération de la surface de l'epithelium olfactif chez l'homme, après un endommagement infectueux superficiel, il semble exister une migration des noyaux des cellules olfactives vers la couche superficielle, induisant peut-être à l'accroissement des proéminences etc. à l'aide de structures comparables à des centrioles.

LITERATURE

ADRIAN, E. D.: The action of the mammalian olfactory organ. J. of Laryng. Otol. Jan. 1956.

ADRIAN, E. D.: Sensory discrimination with some recent evidence from the olfactory organ. Brit. Med. Bull. 1955, P. 1534.

ALLISON, A. C. and WARWICK, R. T. T.: Le neuro-epithelium olfactive. Acta anat. Basel, Tome IV, fasc. ½ 1947.

ALLISON, A. C. and WARWICK, R. T. T.: Quantitative observations on the olfactory system of the rabbit. Brain, London; Vol. 72, P. 186—197 (1949).

ALTHAUS, J.: Physiology and Pathology of the olfactory nerve. Lancet I, P. 813, 1881.

BAKKER, TJ.: Orgaan van Jacobson bij onze huisdieren (Proefschrift) 1939, Utrecht.

BAUD, C. H. A.: La Texture protofibrillaire du neurite. Acta Anat. Basel, Tome 10, P. 461—463, 1950.

BAUER, TH. and BECK, O.: Atlas der Histopathologie der Nase und ihre Nebenhöhlen, Curt Kabitzsch Leipzig, 1924.

BEN GEREN, B. and SCHMITT, F. O.: Electron microscop. studies of the Schwann cell and its constituents with particular reference to their relation to the axon. Fine Structures of cells. Symposium held at the VIII-th. congress of cell biology, Leiden 1954.

BERGER, L. LUC et RICHARD: L'esthesioneuro-epitheliome olfactif. Bull. Assoc. fr. cancer XIII, 410—429, 1924.

BERGER, L. LUC et RICHARD: L'esthesioneurocytome olfactif. Bull. Assoc. fr. cancer XV 404, 1926.

BLOOM, G. V.: Studies on olfactory epithelium of toad and frog. Zschr. Zellforsch. Bd. 41, P. 89—100, 1954.

BLOOM, G. V. and H. ENGSTRÖM: The structure of the epithelium surface in the olfactory region. Exper. cell Res. Vol. 3, P. 699—701, 1952.

BLOOM, G. V. and H. ENGSTRÖM: Interciliary structures in the epithelium of the uppermost respir. tract. Ann. Otol. Vol. 62 P. 15, 1953.

BOUIN et PRENANT: Traité d'Histologie. Tome II Histologie et Anatomie microscopique. L'organe olfactif, P. 518—526. Paris Masson et cie. 1911.

BOURNE, G. H.: Alkaline phosphatase in taste buds and nasal mucosa; Nature, I 1948, Vol. 161, P. 445.

BOWMAN and TODD: Physiological anatomy and physiology of man. Vol. II, P. 5—7, 1847.

BRUNN, A. VON: Untersuchungen über das Riechepithel. Arch. mikrosk. Anat; Bd. 11, S. 468—478, 1875.

BRUNN, A. VON: Weitere Untersuchungen über das Riechepithel und sein Verhalten zum N. olfact. Arch. mikrosk. anat.; Bd. 17, S. 141—151, 1880.

BRUNN, A. VON: Beiträge zur Mikrosk. Anatomie der menschlichen Nasenhöhle. Arch. mikrosk. anat.; Bd. 39, S. 632—651.

BRUNN, A. VON: Zwei mikrosk. Präparate vom Riechepithel eines Hingerichteten. Anat. Anz.; Jena.; Verh. anat. Gesellschaft, S. 133, 1889.

CISOFF: Zur Kenntnis der Regio olfactoria. Zentralb. f. inn. Med. B. 44, S. 689, 1874.

Colosanti: Untersuchungen über die Durchschneidung des Nervus olfactorius bei Fröschen. Arch. Anat. und physiol. S. 469, 1875.
Couteaux, R.: Neuro-filaments et neuro-fibrilles dans les fibres nerveuses de la Sangsue. Electron microscopy. Proceedings of the Stockholm Conference, Sept. '56.
Dalton, A. J.: Electron micrography of epithelial cells of the gastro-intestinal tract and pancreas. Amer. J. Anat. Vol. 89, P. 109—133, 1951.
Dalton, A. J. and Felix: Cytologic and cytochemical characteristics of Golgi substance of epithelial cells of the epididymis in situ, in homogenates and after isolation. Amer. J. Anat. Vol. 94, No. 2, P. 171, 1954.
Disse, J.: Die erste entwickelung des Riechnerven. Anat. Hefte, Wiesbaden, Bd. 9, 1897.
Ecker, A.: Über die Geruchschleimhaut des Menschen. Zschr. Wiss. Zoöl. B. 8, S. 303, 1856.
Eckhardt: Uber die Endigungsweise des Geruchsnerven. Beitr. Anat. Physiol., Heft I, S. 77, 1855.
Eggston, A. A. and Wolff, D.: Histopathology of the Ear, Nose and Troat. Baltimore. The Williams and Wilkins company 1947.
Ehrlich, P.: Uber die Methylen blau reaction der Lebenden Nerven substanz. Dtsch. med. Wschr., Jhrg 4, 1886.
Engström, H.: The structure of tracheal cilia. Acta oto-laryng. Stockholm, Vol. 39, P. 363—366, 1951.
Engström, H. and Wersäll: Nutritive cellular system around the haircell, in the organ of Corti. Ann. of Otol. Rhinol; St. Louis, Vol. 62, P. 507, 1953.
Engström, H. and Wersäll: Some principles in the structure of vibratile cilia. Ann. of Otol. Rhinol. St. Louis, Vol. 61, P. 1027, 1952.
Engström, H. and Sjöstrand, F. S.: The structure and innervation of the cochlear haircells. Acta otolaryng. Stockholm; Vol. 44, P. 490—501, 1954.
Exner, S.: S. ber. Akad. Wiss. Wien. Math. Naturw. Kl. Abt. III, Bd 63, S. 44, 1871.
Exner, S.: Fortgesetzte Studiën über die Endigungsweise des Geruchsnerven. S. ber. Akad Wiss. Wien. Math. Naturw. Kl. Abt III, Bd. 76, S. 173, 1877.
Fawcett, D. W. and Porter, K. R.: A study of the fine structure of ciliated epithelia. J. Morph, Vol. 94, P. 221—283, 1954.
Findlay, J. W.: A research into the histological structure of the olfactory organ. Normal and Pathological Human and Compar. J. of Anat. Physiol., London. Vol. 28, 1894.
Fisher, E. R.: Neuroblastomas of the nasal fossa. Arch. of Path. 60, 435—439, 1955.
Frühwald, V.: Die Folgen des einseitigen Nasen Verschlusses auf die Riechschleimhaut und auf den Bulbus und Tractus olfactorius. Arch. Ohr, Nase und Kehlk.hk.; Bd. 139 S. 153—173, 1935.
Gasser, H.: Olfactory nerve fibres. J. Gen. Physiol. Vol. 39 No. 4, 1956.
Gerebtzoff, M. A. and Shkapenko, G.: Arch. internat. Physiol. Tome 59 P. 423, 1951.
Gerebtzoff, M. A. et Shkapenko, G.: Recherches sur le pigment de la muqueuse olfactive. Assoc. Anat., 38me Réunion, Nancy 1951. Tome 68, P. 511, 1952.
Grassi, B. and Castronovo, A.: Beitrag zur Kenntnis des Geruchsorgans des Hundes. Arch. mikrosk. Anat. Bd. 34, S. 385, 1889.
Gros Clark, W. E. le and Warwick, R. T. T.: The pattern of olfactory innervation. J. Neurol.; London. Vol. 9, New series. P. 101—111, 1946.
Gros Clark, W. E. le and Warwick, R. T. T.: Projection of the olfactory epithelium on the olfactory bulb. Nature. Vol. 165, P. 452—453, 1950.
Gros Clark, W. E. le: The projection of the olfactory epithelium on the olfactory bulb in the rabbit. J. Neurol., London. Vol. 14, P. 1, 1951.
Gros Clark, W. E. le and Meyer, M.: The terminal connections of the olfactory tract in the rabbit. Brain, Vol. 70, P. 304, 1947.

Gros Clark, W. E. le: Observations on the structure and organization of olfactory receptors in the rabbit. Yale J. Biol. Vol. 29, No. 2 P. 83—94, 1956.
Groth: Der Ursprung der Riechzellen neuroblasten und ihre erste Entwickelung bis zur Ausbildung der Riechnervenanlage beim Kaninchen. Zschr. mikrosk. anat. Forsch. Leipzig; Bd. 43. S. 207—233, 1938.
Hartmann, J. F.: Mitochondria in cell bodies of hypoglossal nucleus and spinal ganglia following section of nerves. Anat. Rec. Philadelphia. Vol. 103, P. 541—542. 1949.
Hartmann, J. F.: Mitochondria in neurotomy. Anat. Rec. Philadelphia; Vol. 100, P. 49—56, 1948.
Hartmann, J. F.: Electron optical study of central nervous system. J. of Comp. Neurol. Philadelphia Vol. 99, P. 201—224, 1953.
Hartmann, J. F.: E.M. of motor nerve cells following section of axones. Dep. of anatomy University of Minnesota Minneapolis, Anat. Rec. Philadelphia, Vol. 118, I, P. 19—35, 1954.
Harven, E. de and Bernhard, W.: E.M. etude de l'ultra structure du centriole chez les vertebrès Zeitschrift für Zellforschung und mikrosk. anatomie. Bd. 45, P. 378—379, Springer Verlag 1956/57.
Hennebert, P. E.: L'olfaction. Acta oto-rhino-laryng. Belg. Fasc. 2. 1953.
Hippocrates: Des chairs ou commencement de l'homme. Trad. Cardeil et Coray.
Hirsch and Rinkel, v. Thiel, v. d. Broek: Symposion: Die Rolle der Golgi-Körper bei der Bildung von Zellproducten. Comptes Rendus de la Société Néerlandaise de Zoölogie. 1939. Arch. Néerl. Zoöl. Tome IV, P. 2—3, 1940.
His: Uber die Entwickelung des Riechlappens u.s.w. Anat. Anz. Jena; Verh. anat. Ges. B. 3, S. 63—67, 1889.
Holmfeld, C. D.: Bygningen af Regio Olfactoria, Nord. Med. Ark. B. 15, P. 1—18, 1883.
Hopkins, A. E.: The Olfactory receptors in vertebrates. J. comp. Neurol. Philadelphia. Vol. 41, p. 253—289, 1926.
Hutchinson: A remarkable case of change of complexion with loss of the sense of smell. Am. J. Med. Sc., Ns., Vol. 23 P. 146, 1852.
Kallius, F.: Handbuch der Anat. Hrsg. von Bardeleben Bd. 5, S. 5—115, 1905.
Kolmer, W.: Handbuch der mikrosk. Anat. Hrsg. v. Möllendorf. Bd. 3 Teil I, P. 192, 1927.
Lams, H.: Le neuro-epithelium olfactif. Acta Anat. Basel. Vol. 4, fasc. 1/2 1947.
Lams, H.: Recherches sur la vascularité de certains epitheliums. Le neuro-epithelium olfactif chez les mammifères. Bull. Acad. Méd. Belgique; Tome 5, P. 110, 1940.
Ludford: The cytological action of methylene blue. Arch. exper. Zell. forsch., Jena. Vol. 17, S. 339—359, 1935.
Martin, H. N. Notes on the structure of the olfactory mucous membrane. J. Anat. Physiol. London; Vol. 8, P. 39, 1873.
Masson, P.: Tumeurs humaines. Esthesioneuro-epitheliome olfactif. Deuxième edition. Librairie Maloine S.A. Paris. p. 991—994, 1956.
Mateson, J. F.: The olfactory area and the olfactory receptor process. Ann. N.Y. Acad. Sc. Vol. 58, art. 2; p. 83—95, 1954.
Monroe, B. G.: Electron microscopy of the thyroid. Anat. Rec. Philadelphia; Vol. 116, P. 345—355, 1953.
Müller, A.: Quantitative untersuchungen am Riechepithel des Hundes. Zschr. Zellforsch. B. 41, S. 335—350, 1955.
Nagahara Yoshihiko: Exp. hist.path. studiën über das Geruchsorgan nach der olfactorius durchschneidung. Beitrage des feineren Baus des Geruchsorgans. Transact. Soc. path. Jap. Vol. 5; P. 165, 1940.
Ogle, W.: Anosmia or cases illustrating the physiology and pathology of the sense of smell. Med. chir. Transact. Vol. 53, P. 263, 1870.

PALADE, G.: An electron microscope study of the mitochondrial structure. J. Histoch. Cytoch. Vol. 1, P. 188—211, jan. 1953.

PALADE, G. E. and PORTER, K. R.: The endoplasmic reticulum of cells in situ. Anat. Rec. Philadelphia B. 112 No. 2, P. 370, 1952.

PALADE G. E. and PORTER, K. R.: Studies on the endoplasmic reticulum. J. Exper. Med. Vol. 100 P. 641—655, 1954.

PALADE G. E. and CLAUDE ALBERT: The nature of the Golgi Apparatus. J. Morph. Vol. 85. P. 35, 1949.

PEARSON, A. A.: Development of the olfactory nerve etc. Ann. Otol. Rhinol. St. Louis, Vol. 51, P. 317, 1942.

PEARSON, A. A.: The development of the olfactory nerv. Anat. Rec. Philadelphia, Vol. 79. P. 49, 1941.

PLANEL, H.: Sur les fosses nasales des Rongeurs. Thèse Toulouse 1951.

PORTER, K. R.: Changes in cell fine structure accompanying mitosis. Fine structures of cells. Symposium held at the VIII-th. congress of cellbiology, Leiden 1954.

SCHMITT, F. O.: Structures of nerve axon filaments. J. Exper. Zoöl. Philadelphia. Vol. 113, P. 99—511, 1950.

SCHULTZ, E. W.: Regeneration of olfactory cells. Proc. Soc. Exper. Biol. Med. New York. Vol. 46 P. 41, 1941.

SCHULTZE, M.: Untersuchungen über den Bau der Nasenschleimhaut. Abh. Naturf. Gesellsch. Halle Bd 7, S. 1., 1862.

SCHULTZE, M.: Ueber die Endigungsweise des Geruchsnerven und die Epithelialgebilde der Nasenschleimhaut. Mber. Königl. Preuss. Akad. Wiss. Berlin, S. 504, 1856.

SCHUMACHER, S.: Histologie der Luftwege und der Mundhöhle. Handbuch Hals-Nasen-Ohrenheilk. B. 1 Berlin: Springer 1925.

SJÖSTRAND, F. S.: Electron microscopy of mitochondria and cytoplasmic double membranes. Nature Vol. 171, jan. 1953.

SJÖSTRAND, F. S.: Electron microscopy of cells and tissue. Physical techniques in Biological research. Vol. 3, Chapter VI, edited by Gerald Oster and Arthur Pollister. Acad. Press. inc. New York, 1956.

SJÖSTRAND, F. S. and HANZON, V.: Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas. Exper. Cell. Res. Vol. 7. P. 415—429, 1954.

SJÖSTRAND, F. S. and HANZON, V.: Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy. Exper. cell. Res. Vol. 7, P. 393—414, 1954.

SJÖSTRAND, F. S. and RHODIN, J.: The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. Exper. Cell. Res. Vol. 4, P. 426—456, 1953.

SLOTWINSKY, J.: Etude cytologique comparée du caractère de la sécrétion des glandes olfactives de Bowman chez l'homme et chez les mammifères, rongeurs et insectivores. Compt. Rend. Soc. Biol. Paris. Tome 115. P. 1269, 1934.

SLOTWINSKY, J.: Sur l'appareil reticulaire interne de Golgi dans les glandes olfactives de Bowman chez les mammifères. Compt. Rend. Soc. Biol. Paris Tome 120. P. 462, 1935.

SLOTWINSKY, J.: Sur le caractère de la sécrétion des glandes olfactives de Bowman chez les mammifères. Compt. Rend. Soc. Biol. Paris, Vol. 108, P. 599, 1931.

SLOTWINSKY, J.: Sur le caractère de la sécrétion des glandes olfactives de Bowman chez les mammifères. Compt. Rend. Ass. Anat. 27e Réunion. P. 462—467, 1932.

SMITH, C. G.: Regeneration of sensory olfactory epithelium and nerves in adult frogs. Anat. Rec. Philadelphia. Vol. 109. P. 661, 1951.

Smith, C. G.: Incidence of Atrophy of the olfactory nerves in man. Arch. Otol. New York, Vol. 34, P. 535, 1941.
Smith, C. G. and Graigie, E. H.: The association of a pathological change in the olfactory nasal mucosa with the occurrence of stunted olfactory bulbs of albino rats. Arch. Otol. New York, Vol. 25, P. 131, 1937.
Stricht, O. van der: Le neuro-epithelium olfactif et sa membrane limitante interne. Mem. couronn. Acad. Méd. Belg. 2me série, Tome 2, P. 20, 1909.
Stone: Regeneration from surviving retinal pigment in grafted adult salamander eyes. Anat. Rec. Philadelphia; Vol. 106, P. 89—109, 1950.
Suchannek, H.: Differential diagnostische Merkmale zur Unterscheidung zwischen normalen und pathol. menschlichen Riechepithel resp. respirator. Flimmerepithel. Zschr. Ohr.hk. Wiesbaden; Bd. 22, S. 4—10, 1892.
Suchannek, H.: Beiträge zur mikroskop. Anatomie der menschlichen Nasenhöhle, speziell der Riechsleimhaut. Zschr. Ohr.hk. München; B. 24, 1893.
Takata, N.: Riechnerv und Geruchsorgan. Arch. Ohr. Nas. Kehlk.hk. Bd. 121, S. 31—78, 1929.
Versteeg, N.: Onderzoekingen over de reuk, Dissertatie Leiden, 1956.
Vinnikov, I. A. A.: Degenerative and restorative processes in the olfactory organ in mammals. Bulletin of experimental Biology and Medecine 42 (11), Nov. 1956.

STELLINGEN

I

Er worden bij de kat twee reukepitheel-typen gevonden: één met en één zonder de membrana limitans olfactoria. De eerste is achter in de neus gelegen en waarschijnlijk selectief gevoelig voor o.a. vlees- en visgeur.

II

De regeneratie van de oppervlaktestructuren van het reukepitheel van de kat komt overeen met de embryonale ontwikkeling.

III

Bij de regeneratie van het reukepitheel van mens en kat heeft een migratie van reuk- en/of steuncelkernen plaats.

IV

Het secreet en de pigmentgranula in de klieren van Bowman zijn van belang bij het reukproces van de mens en de kat.

V

Bij een tympanoplastiek is het noodzakelijk om het gehele middenoor en wel speciaal de gehoorbeentjes en vensters te inspecteren en te saneren.

VI

In verband met dreigende binnenoordoofheid verdient het aanbeveling om bij een chronische otitis media niet te lang te wachten met een sanerende c.q. tympanoplastische operatie.

VII

Er is reden om aan te nemen, dat de remming van de gonadotroop hormoon productie in de hypophyse door oestrogene stof plaats vindt door een inwerking van deze stof op de hypothalamus.

VIII

De problematiek van de toneelspeler heeft overeenkomst met die van de adolescent.

IX

De blijvende veranderingen aan de vingers na een febris rheumatica zijn principieel verschillend van de gewrichtsafwijkingen bij rheumatoïde arthritis.

X

Een hechte samenwerking tussen anatoom-embryoloog en klinicus zal het inzicht in de normale en abnormale topographische problemen verruimen.