OLFACTORY BULB INCREASES MARKER PROTEIN IN OLFACTORY RECEPTOR CELLS¹

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Abstract

Olfactory mucosa was excised from the heads of embryonic day 15 (E15) rat fetuses, prior to the appearance of olfactory marker protein (OMP), and cultured with or without the presumptive olfactory bulb. Specific immunohistochemical staining for the OMP was detected in both groups of explants 6 days after explantation. At no time do the supporting cells and basal cells show positive OMP staining. Results of radioimmunoassay on the two groups of cultures showed that about twice as much OMP was present in organ cultures grown with presumptive olfactory bulb. Estimates of the area of olfactory mucosa in the explants revealed no significant differences between those cultured with or without the bulb. Cell counts revealed that the increased amount of OMP in explants with the bulb was due to a higher number of OMP-positive receptor neurons. Explants of olfactory mucosa cultured with other tissues (cerebrum, cerebellum, cervical spinal cord, and heart) contained approximately the same amount of OMP as explants of mucosa alone; i.e., these other tissues did not enhance OMP levels. Similarly, there was no enhancement of OMP level when the olfactory mucosa was separated from the bulb by a Millipore filter of 0.45 μm pore size. Thus the results of the study indicate that the presumptive olfactory bulb specifically increases OMP level by a mechanism that requires direct contact between the receptor cells and bulb.

Olfactory marker protein (OMP) is a low molecular weight, acidic protein found exclusively in receptor neurons of the primary olfactory pathway (Margolis, 1972; Keller and Margolis, 1976). It is synthesized in the perikaryon and transported both to the dendrite and along the axon to its termination in the glomerular layer of the olfactory bulb (Margolis and Tarnoff, 1973). This protein has been demonstrated immunohistochemically by techniques using fluorescein-labeled antibodies (Hartman and Margolis, 1975) and peroxidase-antiperoxidase-labeled antibodies (Monti Graziadei et al., 1977; Graziadei et al., 1979; Farbman and Margolis, 1980; Miragall and Monti Graziadei, 1982). Evidence from immunological studies indicates that it has a very wide species distribution, being present in fishes, amphibians, and

mammals including man (Keller and Margolis, 1975). The functions of OMP are yet to be elucidated, although tests reveal that it does not exhibit the enzyme activity of aldolase, acid phosphatase, amylase, lipase, alkaline phosphatase, or lactate dehydrogenase (Margolis, 1980).

Earlier studies on developing olfactory mucosa have shown that OMP was first demonstrable immunocytochemically on the 18th embryonic day in rats (Farbman and Margolis, 1980) and on the 14th embryonic day in mice (Farbman and Margolis, 1980; Monti Graziadei et al., 1980). This time period is when receptor neurons first establish synapses with their target cells in the bulb, and it was suggested that synapse formation may influence OMP synthesis (Farbman and Margolis, 1980). However, OMP may be synthesized by receptor cells that do not synapse with their usual targets. In mice in which the olfactory bulb was removed unilaterally, the reconstituted receptor cells have been shown to grow into the cerebral cortex and form glomeruli and synapses with cells in that region. These receptors also synthesize OMP (Graziadei et al., 1979; Graziadei and Samanen, 1980). Although the immunohistochemical technique has demonstrated the presence of OMP in these neurons and ectopic glomerular structures, no quantitative study has been done to determine whether there are differences in

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the amounts of OMP in an olfactory receptor population with or without a normal target. The present study utilized the organ culture system in which olfactory receptor cells can grow and differentiate from a primitive cell type to a mature state (Farbman, 1977). Explants of olfactory mucosa with the bulb or other tissues were cultured and assayed for OMP using radioimmunoassay (RIA) and immunohistochemistry. Experiments were also devised to explore the mechanism by which the olfactory bulb enhances OMP level in receptor cells.

Materials and Methods

Pregnant rats (from Holtzman Co., Madison, WI) were killed by ether overdose when their embryos were at stage E15 (the day when the dams were sperm positive was E1). Fetuses were removed under sterile conditions and decapitated, and the olfactory mucosa (OM) lining of each olfactory pit was excised. Each embryo yielded two explants, which measured about 1 mm $\times$ 1 mm. OM explants from these fetuses were cultured either alone or *en bloc* with most of the excised presumptive olfactory bulb (POB). The region of the brain closest to the roof of the olfactory pit was considered the POB. At this stage the POB is separated from the olfactory epithelium by a narrow band of mesenchyme. Other groups of explants included OM separated from the POB and then recombined in culture, and OM cultured with pieces of cerebrum, cerebellum, cervical spinal cord, or heart. In explants where the OM was separated from the POB, an incision was made between these two regions such that the POB fragment contained very little connective tissue. The piece of cerebrum used in culture was the region immediately posterior to the middle meningeal artery. Another group of explants was cultured in which a 25- μ m-thick Millipore filter of 0.45 μ m pore size was placed between the OM and one to three pieces of POB. Instead of Millipore filter some explants had Nucleopore filters (3 μ m pore size) placed between the OM and three fragments of POB. All explants were placed on collagen-coated Millipore filters supported by a stainless steel grid platform in a Falcon culture dish. The culture medium was a serum-free medium consisting of Waymouth's medium 752/1 (Grand Island Biological Co., Grand Island, NY) supplemented with 0.30 mg/ml of L-ascorbic acid and 0.10 mg/ml of gentamicin (Schering Corp., Kenilworth, NJ). Cultures were grown in an atmosphere of 5% CO₂ in humidified air at 35°C.

Some embryos were decapitated and their heads were fixed in Bouin's fixative. These were processed in paraffin and stained with hematoxylin and eosin for routine histological examination.

Radioimmunoassay. A solid phase RIA procedure (Hirsch and Margolis, 1979) was used to estimate the amount of OMP synthesized by the explants.³ After the appropriate growth period, cultures were pooled and homogenized in 100 mM Tris buffer, pH 8.0. For every assay of cultures, a standard curve was run simultaneously.

The RIA was performed in test tubes each containing 0.15 mmol NaCl, 0.01 mmol Tris-HCl, pH 7.4, 10 mg of bovine serum albumin, about 2000 cpm [³H]OMP, the test sample, and an appropriate volume of latex beads (0.6 μ m diameter) (Sigma Chemical Co., St. Louis, MO) either coated with preimmune or OMP antiserum, all in a final volume of 1 ml. The reaction mixture was incubated at 37°C in a shaker for 1 hr and was then left overnight at room temperature. The following day, the incubating mixture was vacuum filtered; the latex beads were collected on Millipore filters (0.45 μ m pore size), dried, and transferred to a scintillation vial to be counted. Radioactivity was determined in the presence of 5 ml of toluene scintillation fluid containing 4 gm/liter of PPO and 0.4 gm/liter of POPOP in a Beckman LS7000 scintillation counter. The lowest counts obtained were those reaction mixtures containing latex beads coated with preimmune serum. The average of such a pair of duplicates was subtracted from all counts to correct for unspecific activity. The reaction mixtures which contained only [³H]OMP (and no competing known amounts of OMP) gave counts equivalent to 100% binding. After appropriate calculations, the values obtained were plotted on semilog graph paper. Figure 1 is a graph of a typical RIA standard curve. The reliability of the standard curve was tested as follows, by assaying for OMP in 25- and 50- μ l volumes of a 1% extract (w/v) of adult rat olfactory bulbs in 100 mM Tris buffer, pH 8.0, for each standard assay. The scintillation counts for the two fixed volumes of bulb extract were reproducible for each standard curve. These two volumes were chosen because they fell within the middle region of the curve. Similarly, to ensure accuracy, a sufficient number of explants was pooled that the amount of OMP present was always near the middle region of the standard curve. The number of explants pooled for each group ranged between 20 and 45. As a control, 20 explants of E15 OM and POB (i.e., at time zero) were also pooled and tested for OMP in each assay. The amount of OMP obtained from different groups of explants was expressed in terms of the quantity found in a 7-day explant of OM and POB *en bloc*. Assays of the cultures showed wide variations from each other. However, these were most likely due to biological variations and not inaccurate experimental procedures because assays of the 25- and 50- μ l aliquots of rat olfactory bulb gave reproducible results for each standard curve (cf. above). Because OMP is a specific protein and is demonstrable in measurable amounts *only* in olfactory receptor cells, it was reasonable to express the results in terms of the amount of OMP per explant, instead of more traditional terms such as the ratio of OMP to total protein by weight. Furthermore, the total amount of protein in an explant of OM combined with any tissue *en bloc* will always be more than an OM explant alone even though the total amount of olfactory epithelium in all groups is approximately the same.

For the sake of convenience the amount of OMP in an explant of OM and POB grown for 7 days was used as a reference. Other points are expressed as ratios of this reference value, arbitrarily set at 1.0.

Immunohistochemistry and cell counts. After periods varying from 6 to 10 days, explants were fixed in Bouin's

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Figure 1. Standard radioimmunoassay curve. To test its reliability, 25 and 50 μ l of 1% extract adult olfactory bulbs (OB ext.) were assayed for OMP for each standard assay. The counts and, consequently, the percentage binding for these two volumes were reproducible for each standard curve.

fixative and processed in paraffin for routine histological examination and immunohistochemical staining. They were sectioned at 7 μ m and stained with hematoxylin and eosin for histological examination. Appropriate sections were selected for immunohistochemical staining. These were placed on slides and deparaffinized to water. The peroxidase-antiperoxidase (PAP) indirect immunocytochemical technique (Sternberger, 1979) was then applied to reveal the presence of OMP. The specific immunohistochemical staining procedures have previously been described in detail (Farbman and Margolis, 1980). The PAP procedure involves sequential application of the following solutions at 37°C: normal undiluted rabbit serum (15 min); goat anti-OMP serum (diluted 1:100, incubated 45 min); rabbit anti-goat serum (Grand Island Biological Co.) (diluted 1:50, incubated 45 min); and goat PAP serum (N. L. Cappel Laboratories, Cochranville, PA) (diluted 1:200, incubated 30 min). Between each serum treatment, the sections were washed for 10 min in phosphate-buffered saline (PBS) at room temperature. The diluent for anti-OMP is made up of one part PBS and one part normal rabbit serum. After incubation with PAP and washing with PBS, the specimens were incubated for 5 min at room temperature in a solution made up of 50 mg of 3,3'-diaminobenzidine tetrahydrochloride and 600 μ l of 3% hydrogen peroxide dissolved in 100 ml of 0.05 M Tris buffer. Following this, the sections were counterstained in 0.5% methyl green. To prepare absorbed antisera, aliquots of OMP antiserum were treated with purified OMP. The sequence of steps include repeated centrifuging and addition of more aliquots of OMP; final samples were washed with buffered saline (Farbman and Margolis, 1980).

Control specimens of the PAP procedure were those incubated either with normal goat serum or antiserum preabsorbed with OMP as described.

For cell counts, explants were serially sectioned at 7 μ m and stained for OMP to obtain a quantitative esti-

mate of the number of OMP-positive cells. To avoid the possibility of counting the same OMP-positive receptor more than once, alternate sections were examined. Only cells that showed immunoreactivity in both the perikarya and dendrites were counted. Because only alternate 7- μ m sections were examined, the number of OMP-positive receptors obtained per explant is almost certainly an underestimate of the absolute count of receptors that have this protein. However, all sections were counted this way so that errors should be cancelled out.

To obtain an estimate of the area covered by the olfactory epithelium in both OM and POB *en bloc* and OM alone explants, the length of the epithelium was measured with an ocular reticle on every 20th section. The average of all measured lengths of olfactory epithelium was calculated. The sections were measured on an Olympus BH light microscope fitted with an objective and ocular, each of $\times 10$ magnification. The reticle was marked with squares 1 mm wide and was calibrated so that 1 mm = 100 μ m.

Results

At E15 the receptor cells were relatively undifferentiated, although axonal processes had formed and were emerging through the basal lamina into the mesenchyme. After about 7 days' culture, discrete nerve bundles could be seen coursing through the mesenchyme (Figs. 2 and 3). In cultures where the POB was absent, the nerve bundles often terminated in neuroma-like structures. In explants where olfactory epithelium was combined with the POB, nerve bundles could be traced into the brain tissue, terminating in a glomerular-like region (Fig. 3a). The cells on the periphery of the brain tissue, which were larger and stained lighter than the other cells, were often aligned in a fairly distinct layer (Fig. 3a). Generally there was better growth when the olfactory epithelium was on the upper surface of the explant.

Radioimmunoassay. All RIAs conducted on E15 spec-

Figure 2. OMP-positive receptor neurons in the olfactory epithelium of a 7-day OM alone explant. Large numbers of axons (arrows) can be observed in the connective tissue (CT). Magnification $\times 410$.

Figure 3. Seven-day-old OM and POB explant. A, Hematoxylin and eosin stain showing POB on the right and olfactory epithelium (OE) on the left. Cells on the periphery of the POB are larger and stain less intensely (arrow). B, Section stained for OMP. Axons containing OMP (a) can be seen coursing from the OE and terminating next to the POB in a glomerular-like region (g). C, Control section. Normal goat serum was used in place of OMP antiserum. No staining is observed anywhere on the section. Some cartilage (c) had differentiated in the explant. A, B, and C were taken from the same explant. Magnification $\times 80$.

Figure 4. Comparison between OMP ratios of OM and POB *en bloc* and OM alone. The amount of OMP in each explant is expressed as a ratio of OMP in an OM and POB explant cultured for 7 days. The points represent averages of two to four batches of explants each containing a minimum of 20 explants. Explants of different litters were assigned to groups in a randomized fashion. The error bars represent the range of values obtained; i.e., the lower and upper limits of the bar indicate the minimum and maximum OMP ratios for that point.

Figure 5. Comparison between OMP ratios of OM and POB *en bloc*, and A, OM and cerebrum; B, OM and heart; C, OM and POB separated and recombined; and D, OM and POB separated by Millipore filter of 0.45- μ m pore size. Some explants in D consist of one piece of OM cultured with three fragments of POB. The OMP ratio at each time point is an average value of two to five batches each containing a minimum of 20 explants. Explants of different litters were assigned to groups in a randomized fashion. The OM + POB curve from Figure 4 is reproduced in this figure for comparison. The error bars represent the range of value obtained.

imens showed the absence of OMP. The respective OMP ratios obtained from groups of explants harvested at different times in culture were plotted on graphs. Figure 4 shows that those cultured with the POB *en bloc* had more OMP. The rate of increase in the amount of OMP in this group was greatest between the 5th and 6th day in culture, after which the level remained relatively constant. In explants of OM alone, there was consistently less OMP at all time points after the 6th day. By the 7th day, the amount of OMP in explants of OM and POB was about twice that of OM alone.

The OMP ratios of OM cultured with fragments of cerebrum and heart from 4 to 10 days demonstrated that the amount of OMP attained was always less than in OM and POB (Fig. 5, A and B). Seven-day explants of

OM cultured with cerebellum or cervical spinal cord also had about half the amount of OMP in explants of OM and POB (Table I). The evidence suggests that the enhancing effect is a specific influence from the olfactory bulb, which is not observed with other parts of the central nervous system tested. By utilizing a 25- μ m-thick Millipore filter to separate the mucosa from the bulbs, we attempted to examine the nature by which the enhancement occurs. If the enhancement is triggered by a release of diffusible soluble factor(s) from the bulb, the resulting effect should be an increased amount of OMP. However, if direct cell contact is required, then no increase should be observed. The graph of Figure 5D indicates that the latter suggestion is consistent with experimental results. Moreover, the experiments in which explants of OM and

TABLE I

OMP ratio of groups of explants cultured for 7 days

The average amount of OMP in an OM and POB explant was arbitrarily set at 1.0 and all values of OMP in other groups were expressed as a ratio of this value. The numbers in parentheses indicate the number of batches assayed. Each batch contains from 20 to 45 explants.

Groups of Explants	OMP Ratio
OM + POB <i>en bloc</i>	1.00
OM + POB separated and recombined	0.97 (3)
OM alone	0.51 (3)
OM + Heart	0.33 (3)
OM + Cerebrum	0.44 (3)
OM + Cerebellum	0.45 (3)
OM + Cervical spinal cord	0.58 (4)
OM + 3 pieces of POB, separated by:	
(a) Millipore filter, 0.45 μm pore size	0.53 (3)
(b) Nucleopore filter, 3.0 μm pore size	0.46 (5)

POB were first separated and recombined in culture showed results similar to those in which mucosa and bulb were cultured *en bloc* (Fig. 5C). To test the possibility that a diffusible factor may be bound by the Millipore filter, some cultures were done using Nucleopore filters (made of a polycarbonate compound) in place of the Millipore (made of a cellulose). The results were essentially similar; i.e., there was no increase of OMP levels when a porous filter was inserted between the OM and the POB in the culture.

Immunohistochemistry and cell counts. The immunohistochemical staining revealed that a positive reaction was seen in the soma, dendrite, and axon of a few receptor cells by the 6th day of culture. More OMP-positive cells were found on 7-day-old explants. The reaction product was never seen in the supporting or basal cells. In cultures of OM and POB *en bloc*, reaction product for OMP was found in the region where the axons terminated next to the POB tissue (Fig. 3b). The nerve bundles were usually stained less intensely than the perikarya of the receptors. It was also found that OMP was demonstrable regardless of whether or not the POB was present, although in some cases there appeared to be more OMP-positive cells when a fragment of the brain was present. When normal goat serum or preabsorbed antiserum was used in place of the antiserum, organ cultures of both OM alone and OM with POB *en bloc* consistently showed no staining in the receptor neurons (Fig. 3C).

The cell counts show that the increased amount of OMP in explants of OM and POB *en bloc* was due to a significantly larger number of OMP-positive receptor neurons. Since the increased number of OMP-positive receptors may be a direct result of better general growth resulting in an increase in total epithelial cell number and hence a larger epithelial sheet, we decided to count the number of 7- μm sections spanned by the olfactory epithelium. The results showed that for both groups of explants the number of sections spanned was very close in number (Table II). Moreover, the average length of the epithelium in both groups was also approximately the same (Table II). This indicates that in the OM and POB explant, there is a real increase in the number of OMP-positive receptors per unit area of OM.

TABLE II

Comparison between the number of OMP-positive cells on OM and POB explant and an OM explant

Alternate 7- μm sections were examined, and the number of sections spanned by the epithelium in each explant was counted. For every 20th section in each group, the length of the epithelium was measured on a light microscope using a reticle of squares with sides 1 mm. Each millimeter was calibrated so that 1 mm = 100 μm . The average of all measured lengths of olfactory epithelium was calculated. *n* refers to the number of explants examined.

	Average No. of OMP-positive Cells	No. of Sections per Explant	Average Length of Olfactory Epithelium μm
	$p < 0.005$		
OM alone	220 $\pm$ 103 (<i>n</i> = 14)	275 $\pm$ 42 (<i>n</i> = 14)	1320 $\pm$ 260 (<i>n</i> = 6)
OM + POB	503 $\pm$ 171 (<i>n</i> = 15)	259 $\pm$ 38 (<i>n</i> = 15)	1290 $\pm$ 70 (<i>n</i> = 6)

Discussion

The results of this study show clearly that olfactory receptor neurons from E15 rat embryos can undergo differentiation and synthesize OMP under suitable organ culture conditions in serum-free medium. The receptor neurons were able to synthesize OMP even in the absence of vascularization and innervation from external sources. The POB was shown to have an enhancing or facilitating effect on OMP level, resulting in twice as much OMP in an OM and POB *en bloc* explant compared to OM alone. The increased amount of OMP is due to a higher number of receptors possessing the protein. It is not known, however, whether the increased level of OMP is a result of synthesis or reduced degradation or both. The enhancing effect of the POB appears to be a specific influence and is not observed when substitute tissues are cultured together with the OM. The mechanism by which this occurs probably involves direct cell contact.

In a previous study, organ cultures of differentiating olfactory epithelium did not exhibit any immunohistochemical staining for OMP, even when the antiserum was applied undiluted (Farbman and Margolis, 1980). The detection of OMP in the present study may be explained by the following reasons. First, in the previous study, olfactory explants from younger embryos at E12 and E13 were used (Farbman, 1977; Farbman and Margolis, 1980). It is conceivable that the culture conditions employed in the previous experiments were not suitable for the maturation of receptor cells to the point when they would synthesize OMP. In addition, it was found that Bouin's fixative gave better fixation results than Karnovsky's fixative which was used in the previous study. Because the OMP antigen may have been less well preserved, it may not have been detected in those cultures.

One of the factors that has been correlated with the appearance of OMP in mature receptor cells is the formation of synapses. In mice, OMP appears in receptor neurons 1 day after synapses are first observed; that is, at E15 (Farbman and Margolis, 1980; Monti Graziadei et al., 1980). It has been speculated that OMP might play a role in synaptogenesis between the first-order receptors and the second-order mitral and tufted cells (Farbman

and Margolis, 1980). Conversely, it is also possible that formation of a synapse may induce receptor cells to synthesize OMP, possibly by a retrograde trophic mechanism. It is clear from the present study that the presence of synapses is not a prerequisite for the synthesis of OMP, since the protein can be demonstrated in organ culture of OM alone. A recent study by Barber et al. (1982) showed also that contact with the central nervous system is not necessary for expression of OMP. In their experiments, adult rat olfactory epithelium was transplanted as an autograft into the anterior chamber of the eye. Following degeneration of the axotomized neurons, new receptors differentiated and were shown to synthesize OMP 8 weeks after transplantation. Although OMP can be demonstrated in receptors without a target, it does not rule out the possibility that OMP may play a role in synaptogenesis. Further work needs to be done to ascertain this possibility.

In other systems in which nerve cell differentiation has been studied, the target organ of the growing axon often has a major effect on the survival and maturation of the neuronal cells. For example, embryonic motoneurons are unable to survive in the absence of connections with muscle cells (Hamburger, 1934, 1958; Barron, 1943, 1946, 1948; Hamburger and Keefe, 1944; Cowan and Wenger, 1967; Oppenheim et al., 1978). Studies in chick embryos showed that early limb bud ablation resulted in the death of the neurons in the lateral motor column, only about 50% of which is natural cell death (Oppenheim et al., 1978). Similarly, the neurons of the ciliary ganglion, a parasympathetic ganglion, are dependent upon their target tissues for survival (Landmesser and Pilar, 1974; Pilar and Landmesser, 1976). Phenomena of target influence on neuronal maturation have also been observed in the autonomic nervous system (Coughlin, 1975; Dibner and Black, 1976; Dibner et al., 1977; Coughlin et al., 1978). Superior cervical ganglion (sympathetic) grown with target salivary glands showed greater elaboration and directionality of nerve fiber outgrowth than did control explants (Coughlin et al., 1978).

In considering the increased amount of OMP found in explants of OM and POB *en bloc*, it is clear that the presence of the POB enhances OMP level in receptor neurons. The higher amount of OMP is most likely due to a real increase in the number of receptors containing this protein. The number of sections and average length of the olfactory epithelium in both OM and POB *en bloc* and OM alone explants were similar, indicating that the area of the epithelial sheet was almost identical in both groups. Consequently, the number of OMP-positive receptors per unit area was larger in the OM and POB *en bloc* group. The OMP enhancement appears to be a specific influence from the bulb since the effect is not seen in cultures with the cerebrum, cerebellum, cervical spinal cord, or heart. The viability of these other tissues was confirmed by light microscopy. Target-specific influence of this nature has been reported in studies involving the autonomic system (Chamley et al., 1973; Chamley and Dowel, 1975). Nerve growth was significantly higher when sympathetic ganglion explants were grown with heart tissue than with spinal cord. In fact, significantly more nerve fibers grew to the atrium than to the normally

less densely innervated ventricle, clearly demonstrating that the relationship between the neuron and its normal target involves a specific interaction (Chamley and Dowel, 1975).

As early as 1928, Ramon y Cajal had postulated that target cells release diffusible molecules which influence certain activities of the presynaptic cells, such as the direction and rate of neurite growth. It has been confirmed by many studies (e.g., Mobley et al., 1977; Coughlin et al., 1978; Ebendal, 1979; Brookes et al., 1980; Dribin and Barrett, 1980; Henderson et al., 1981; Dribin, 1982), which have demonstrated the release of neuronotrophic factors from the target tissue. However, our study suggests that the specific effect of the olfactory bulb may not be mediated by the release of diffusible factor(s). Experiments in which the OM is separated from as many as three fragments of bulb by two types of very thin porous filters suggest that direct cell contact is required. The Millipore filter presents no barrier to the diffusion of molecules since it is known that compounds with a molecular weight as high as 10^7 easily pass through a 0.1- μm pore (Wartiovaara et al., 1974). On the other hand, the disorderly array of channels in the Millipore filter and the thickness itself prevent any transfilter penetration by receptor axons. In the experiments in which we have substituted a Nucleopore filter (Nucleopore, 12.5 μm thick) with a 3- μm pore size, we obtained the same results; i.e., there was no enhancement of OMP levels. Although it is possible that a putative diffusible molecule may adhere to the Millipore filter, it is less likely that this would be the case with both types of filter because they are made of different materials. Cuschieri and Bannister (1975), in a developmental study of mouse olfactory mucosa, indicated that the diameters of receptor axons vary widely between 0.05 and 2.0 μm . They are in bundles enclosed by fingerlike extensions of Schwann cells. Because of the size and unique arrangement of unmyelinated olfactory axons in clusters, it is not surprising that olfactory nerve bundles could not grow through the channels of a Millipore or Nucleopore filter to reach the other side. We were unable to detect axons within the Millipore filters in light microscopy preparations of explants. Preliminary electron microscopic examination of specimens from the Nucleopore experiment also showed no evidence that axons grew through the filter pores. Among previous studies of embryonic development mediated by direct contact between interacting cells is the induction of metanephric kidney tubules by spinal cord and salivary mesenchyme (Wartiovaara et al., 1974; Saxen et al., 1976). Although the mode of exchange of information between the apposed cell surfaces of the interacting tissues is not yet known, it may involve the passage of molecules or an exchange of surface material. Theories have been proposed which speculate on the cellular binding and recognition events that occur among cells. Sperry's chemoaffinity hypothesis (1963) suggests that a large repertoire of gene products may be required for complementary binding between cells. In contrast, modulation theories (Edelman, 1976, 1983) claim that only a few molecules may be necessary. These molecules generate a wide pattern by alterations in their temporal expression or by undergoing local sur-

face modulation. Whatever the case may be, it is conceivable that in the primary olfactory pathway, an inductive molecule from the POB binds with receptors on the axonal terminations of the primary neurons. This purported molecule may in some way affect the OMP level by triggering an increased rate of synthesis of OMP, a decreased rate of degradation, or both. A recent study by Kream and Margolis (1982) demonstrated that, compared to adult mice, young mice (5 to 6 weeks old) have an accelerated rate of synthesis of OMP, matched by an increased rate of degradation in the bulb region. The resultant effect is a higher OMP-specific activity in younger animals. Whether a similar metabolic situation is also occurring in an OM and POB explant remains to be determined.

From our study, we conclude that the presence of POB in organ cultures specifically results in increased levels of OMP; we propose that the mechanism by which this occurs requires direct contact between receptor cells and POB. Further work is required to explore this aspect of intercellular communication as well as the metabolic processes related to OMP turnover.

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