# STUDIES ON DISPERSION OF RABBIT OLFACTORY CELLS

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## INTRODUCTION

Mechanical, chemical, and enzymatic techniques (4, 6, 8, 14, 18, 19) have been reported for dissociation of cells from liver, kidney, intestinal, ovarian, brain, and embryonic tissues; but a method for obtaining cell suspensions from the olfactory epithelium has not been reported in the literature.

Earlier investigations of olfaction have utilized olfactory tissue without attempting to study individually the cellular constituents of the olfactory epithelium. Research on the olfactory receptor mechanism has concentrated mainly on odorant molecules, anatomical descriptions, and electrophysiological studies (3, 11, 12, 15, 16, 20–22).

This paper describes means for acquisition and handling of cell dispersions from olfactory epithelial tissue. The cell suspensions include the sensing bipolar cells, supporting sustentacular cells, ciliated columnar respiratory cells, and other cells which presumably are of lesser or of no importance in the odor-sensing mechanism. Utilization of these techniques should facilitate isolation of specific cell types; and subsequent biochemical investigations should lead to a better understanding of the biological odor-sensing mechanism.

## MATERIALS AND METHODS

### *Chemicals and Assay Methods*

Protein determinations were by the Lowry method (13) using Armour bovine serum albumin dissolved in buffer (see below) as a standard. Absorbancy was measured at 660 m$\mu$. The assay method for cytochrome oxidase was essentially that of Cooperstein and Lazarow (5). The activated cytochrome oxidase systems were prepared by mixing the test samples, just prior to assay, with sufficient sodium deoxycholate (DOC) (17) to give a final concentration of 1%. Osmotic pressures were determined[1] with a Mechrolab vapor pressure osmometer. Buffers for suspending the cells contained $2.7 \times 10^{-3}$ M KCl, $6.0 \times 10^{-4}$ M $MgSO_4$, $4.0 \times 10^{-3}$ M $Na_2HPO_4$, $1.0 \times 10^{-3}$ M $KH_2PO_4$, and sufficient NaCl (3.4 to $20.4 \times 10^{-2}$ M) to give the desired osmotic properties at pH 7.5. The optimum NaCl concentration in the buffer was $6.8 \times 10^{-2}$ M.

### *Dissection and Cell Dissociation*

The experimental procedure for obtaining suspended olfactory cells has been summarized in Fig. 1

[1] Osmotic pressures determined through the courtesy of Dr. J. S. Lee of the Department of Physiology, University of Minnesota, Minneapolis.



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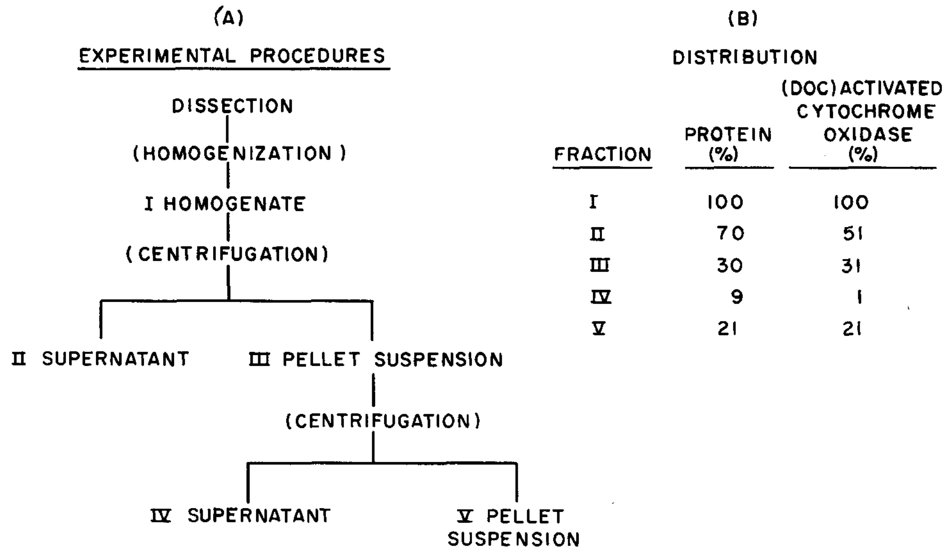


| FRACTION | PROTEIN (%) | (DOC) ACTIVATED CYTOCHROME OXIDASE (%) |
|---|---|---|
| I | 100 | 100 |
| II | 70 | 51 |
| III | 30 | 31 |
| IV | 9 | 1 |
| V | 21 | 21 |

FIGURE 1 Experimental procedures and distribution summary.

A Homogenization was accomplished by 12 complete up and down strokes of the loose-fitting (0.07 mm minimum clearance) Teflon pestle, and centrifugation was done at 700 *g* for 5 min.

B The protein concentration of the homogenate varied from 0.5 to 2.0 mg of protein per milliliter. Based on the homogenate, fraction I, as 100%, fractions II and III represent a recovery of 82% of the enzyme activity and 100% of the protein. Based on the pellet suspension, fraction III, as 100%, fractions IV and V represent a recovery of 71% of the enzyme activity and 100% of the protein.

to facilitate identification of the various fractions as they are discussed throughout the report. The test animals were albino rabbits of domestic stock obtained from a local supplier. The animals were anesthetized with sodium pentobarbital and then decapitated. The nasal cavity was exposed and the septum and entire assembly of turbinate bones were carefully removed, placed in the appropriate buffer and maintained at 4° C. The yellow-brown pigmented olfactory tissue was stripped away from the bony and cartilaginous portions and placed into fresh buffer, 20 to 30 mg/ml, and minced with surgical shears. The minced tissue was homogenized in a loose-fitting Potter-Elvehjem grinder with a minimum clearance of 0.07 mm between the Teflon pestle and the ground glass receptacle. The homogenate containing 250 to 500 mg wet tissue per rabbit was filtered through two layers of surgical gauze into a graduated centrifuge tube and brought to a known volume. Sufficient buffer was added to give a protein concentration between 0.5 and 2.0 mg per milliliter.

The crude cell suspension was centrifuged at 700 *g* for 5 min, and the cells were washed by resuspension of the pellet in fresh buffer followed by another centrifugation at 700 *g*. Cell suspensions for further investigation were then prepared by resuspension of the washed pellet in the appropriate buffer.

### *Identification of Cells from Olfactory Tissue Cell Counting*

The three cell types which are characteristic of the tissue suspensions from olfactory epithelium are the bipolar cells, sustentacular cells, and ciliated columnar respiratory cells. The distinguishing anatomical characteristics of these cells are identified in Figs. 2 and 3.

Since, in many instances, absolute identification of a cell as bipolar or sustentacular was not possible, the two cell types were counted as total number of olfactory cells. (In this paper the term olfactory cells designates a mixture of bipolar and sustentacular cells.) The suspensions were diluted approximately 1:10 with buffer, thoroughly mixed, and applied to the hemacytometer with a disposable pipette. At this dilution a total of 25 to 40 olfactory cells were counted in the center square millimeter section of the hemacytometer, under bright field illumination. All cell dispersions were sampled and counted by at least two different investigators and the average values recorded.

## RESULTS

The effects of various experimental parameters have been studied to determine the best condi-



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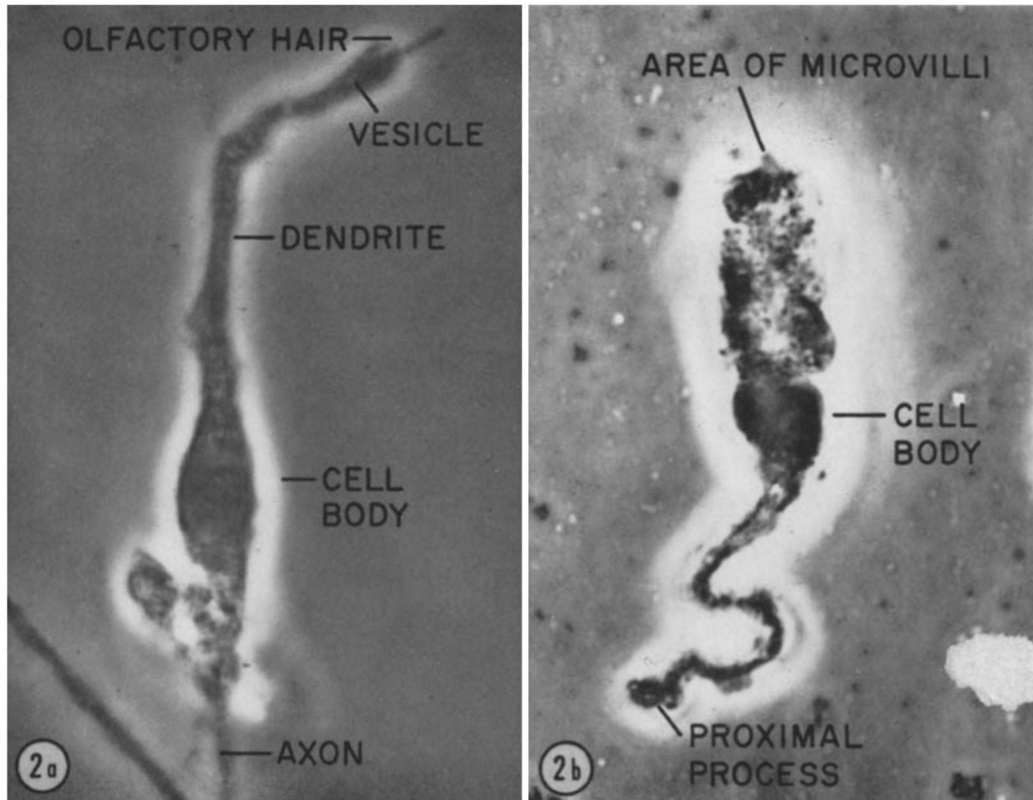


FIGURE 2 Photomicrographs of suspended olfactory cells. Preparation of the olfactory cell suspensions has been described in Materials and Methods. Details were enhanced by addition of 0.25% hematoxylin to the cell suspensions, and the phase-contrast photomicrographs were made from wet preparations of the stained cells. Figure 2 *a* is a photomicrograph of a sensing bipolar cell showing the axon, cell body, dendrite, vesicle, and one olfactory hair. Figure 2 *b* shows the area of the microvilli, cell body, and proximal process of a sustentacular cell. × 1100.

tions for obtaining and maintaining optimum yields of dispersed olfactory cells.

### *Cell Dispersion By Mechanical Tissue Grinding*

The extent of homogenization was varied from 3 to 48 strokes (1 stroke means an up and down cycle). To achieve good cell dispersion, 12 strokes were found to be sufficient.

Mild shaking of tissue pieces with small glass beads was investigated, but found to cause extensive rupture of the olfactory cells. Also, attempts to separate cells of the olfactory epithelium by using proteolytic enzymes (pronase and bromelin) were unsuccessful; the olfactory cells were destroyed.

Cytochrome oxidase, which is found in intact mitochondria (9), was used as a marker for determining the extent of cellular breakage. Since released mitochondria do not sediment at 700 *g* (1, 7, 10), the cytochrome oxidase activity of the 700 *g* sediment, as shown in Fig. 1, is attributable to the tissue cells which were not ruptured during homogenization but were preserved through the washing procedures. The experiments summarized in Fig. 4 provided further evidence of the integrity of the suspended cells. In the absence of the solubilizing agent (nonactivated), the cell suspensions had very little cytochrome oxidase activity; but when deoxycholate was added (activated) to the cell suspensions, cytochrome oxidase activity was in-



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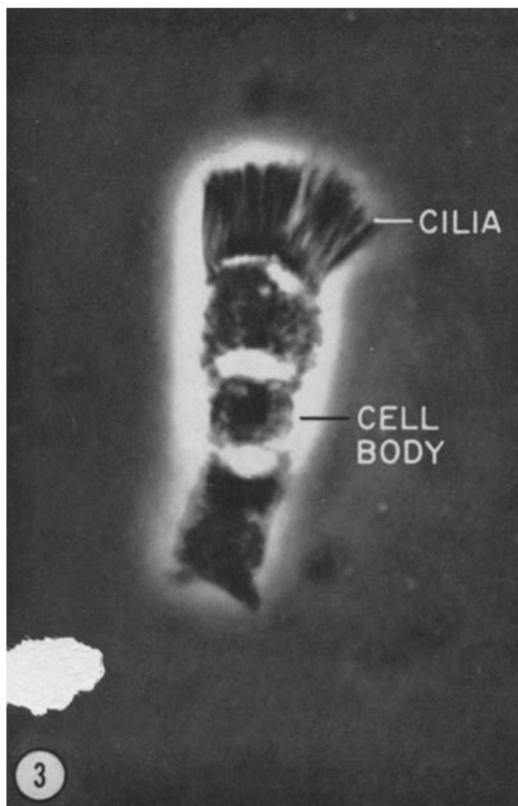


FIGURE 3 Photomicrograph of ciliated columnar respiratory cell. The phase-contrast photomicrograph shows a ciliated columnar respiratory cell from the olfactory epithelium. The cell suspension in phosphate-buffered saline was prepared according to the method summarized in Fig. 1. × 1500.

creased approximately 10-fold. Increased cytochrome oxidase activity has been interpreted to be a result of membrane solubilization by deoxycholate. Addition of deoxycholate, to a 1% level, to cell suspensions III and V increased the cytochrome oxidase activity by 900% whereas addition of deoxycholate to the wash buffer, IV, had essentially no effect.

### *Separation of Contaminating Protein*

Whole cells, debris from broken cells, and soluble proteins contribute to the total protein content of the homogenate. Table I shows that in filtered tissue homogenates approximately 70% of the protein is in the supernatant (noncellular fraction) after centrifugation for 5 min at 700 *g*. About 30% of the pellet protein (9% of homogenate protein) is removed by a second portion of phosphate-buffered saline, and two subsequent washings of the 700 *g* sediment each remove approximately 2.5% of the total protein. Routinely, the suspended cells are washed only twice since unnecessary washings could be expected to cause cell damage and to further extract cellular proteins. The anatomical identity of the olfactory cells is preserved through at least four washing procedures, as observed by light microscopy. However, the extent of cellular damage caused by repeated washings can best be ascertained by biological activity investigations.

Though only a small fraction of the total protein can be attributed to suspended olfactory cells, the total protein content can be used as an indication of the number of olfactory cells in the washed suspensions when the same procedure is rigorously followed. After the tissue has been homogenized and twice washed in the appropriate volume of buffer, each milligram of protein in the cell suspension from 1 to 2 month old rabbits represents approximately $4 \times 10^5$ olfactory cells, and from adult rabbits, $7.5 \times 10^5$ olfactory cells.



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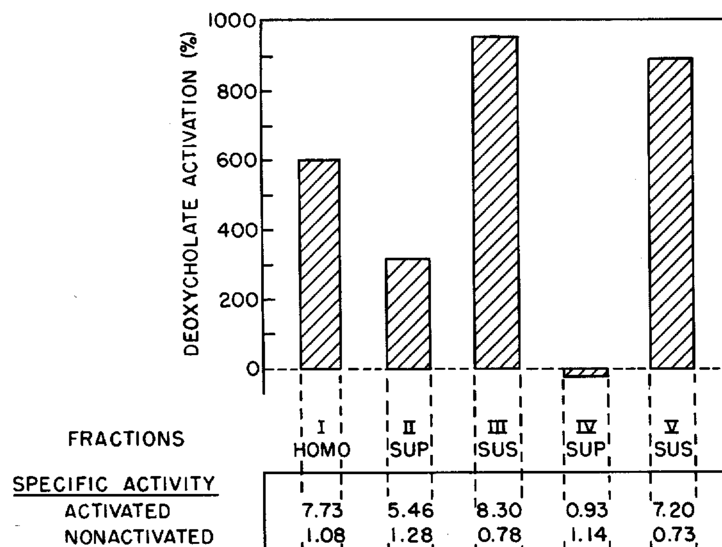


FIGURE 4 Cytochrome oxidase activation. The bar graph shows the per cent activation of cytochrome oxidase due to addition of 1% deoxycholate. Preparation procedures for fractions I to V are shown in Fig. 1. Specific activities were calculated by the formula (5),

$$\text{Specific Activity} = \frac{(\log \Delta A_1 - \log \Delta A_2)\ 2.30}{(T_2 - T_1) \text{ min (protein) mg}}$$

TABLE I

*Washing of Rabbit Olfactory Cells*

| Washings | Number of experiments | Average per cent of total protein homogenate, 100% | | Cell concentration, cells/mg of homogenate protein, | |
|---|---|---|---|---|---|
| | | Supernatant | Pellet | Olfactory | Other particles |
| | | | % | | |
| 1 Centrifuged homogenate | 31 | 68.9 | 31.3 | $128 \times 10^3$ | $3367 \times 10^3$ |
| 2 | 31 | 9.2 | 21.3 | | |
| 3 | 3 | 2.5 | (18.8) | | |
| 4 | 3 | 2.3 | (16.5) | $128 \times 10^3$ | $1343 \times 10^3$ |

Experimental details have been described in Materials and Methods. The values in parentheses were calculated from the supernatant values, assuming complete protein recovery. As a general procedure the olfactory cell preparations were subjected to two washings and then suspended in the appropriate buffered saline.

### *Increasing the Relative Proportion of Olfactory Cells*

OSMOTIC EFFECTS: In vivo, the olfactory cells are closely associated with two important biological fluids, mucus and interstitial fluid. These fluids can be expected to have quite different osmotic properties. Since osmotic pressure values for nasal mucus were not available, the optimum osmotic pressure for a dispersion medium had to be determined. A range of buffers whose osmotic properties varied between those of saliva, another epithelial fluid, at 40 to 180 milliosmols and those of blood at 295 to 300 milliosmols (2) was investigated. Many olfactory cells retain their anatomical identity, as observed by light microscopy, over the



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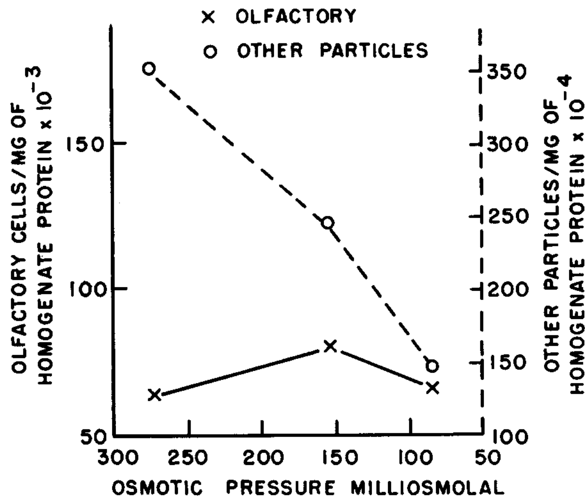


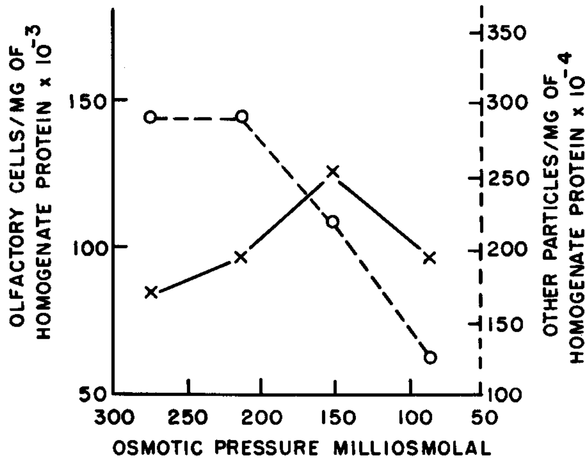


FIGURE 5 Osmotic effects on suspended olfactory cells. The influence of osmotic pressure on the suspended olfactory cells from young (0 to 2 months) and adult ($\geqq$ 4 months) rabbits is shown in Fig. 5 *a* and *b*, respectively. Variations in osmotic pressure are achieved by changing the concentration of sodium chloride. Dispersion details and buffer compositions are explained in Materials and Methods.

entire buffer range, but, as shown in Fig. 5, the yield of olfactory cells and the amount of extraneous (nonolfactory) cellular material in the suspensions were influenced by the osmotic pressure of the suspending buffer. Erythrocytes present in our olfactory tissue homogenates were preferentially ruptured by lowering the osmotic pressure to 85 to 155 milliosmols. Although continued lowering of the osmotic pressure gave increased destruction of red blood cells, there is an optimum osmolal concentration for recovery of olfactory cells. The buffer giving the best yield of suspended olfactory cells was in the 145 to 155 milliosmolal range, and this buffer was routinely utilized for subsequent investigations.

AGE OF RABBITS: During the course of this study, rabbits of varying ages were used. Only about half the number of cells per milligram of homogenate protein (Fig. 5 *a*) were yielded by 1 to 2 month old animals as was found in rabbits 4 months old or older (Fig. 5 *b*). Rabbits up to 3 yr of age were found suitable as long as the animals were free of rhinitis.

## DISCUSSION

Selected experimental parameters associated with



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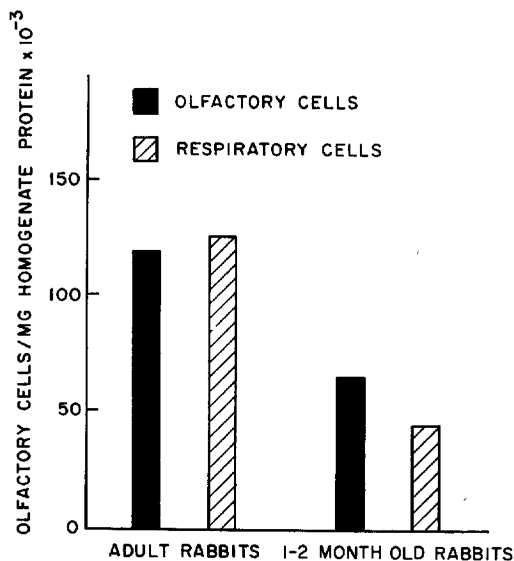


FIGURE 6 Age of rabbits effects yields of suspended olfactory and respiratory cells. Twice washed olfactory cells were suspended in saline containing phosphate buffer, and the cell suspensions were counted under the light microscope with a hemacytometer. Adult rabbits were 4 months or older and weighed at least 2500 gm, whereas the 1 to 2 month old rabbits weighed about 400 to 1200 gm. Values given for adult and young rabbits are the averages of 18 and 15 independent experiments, respectively.

separation and dispersion of cells from the olfactory epithelium have been studied to determine conditions for obtaining maximal yields of suspended olfactory cells.

The cytochrome oxidase experiments summarized in Figs. 1 and 4 provided biochemical evidence that a portion of the harvested cells remained essentially intact through the homogenization and wash procedures. Of the total cytochrome oxidase activity, 20% remained in the suspension of washed cells whereas only about 1% of the original activity (Fig. 1 *b*, fractions IV and V) remained in the supernatant.

Because future investigations of the olfactory cells will include experiments extending for more than 1 day, it was important to determine the fate of the suspended cells when stored. The number of olfactory cells identified by our counting techniques gradually decreased with prolonged storage at 2 to 4°C, but even after storage for 14 days approximately 50% of the olfactory cells retained their identity. Preliminary investigations indicated that the cells retained their morphological identity, as observed by light microscopy, even after freezing to liquid nitrogen temperatures, −195°C and subsequent thawing.

Respiratory cells were found in all cell suspensions and were readily identified by numerous cilia projecting, in parallel, from the distal end of their elongated cell bodies (Fig. 3). As shown in Fig. 6, the number of respiratory cells in the cell suspensions correlated remarkably well with the number of suspended olfactory cells. Such a correlation would not be expected if the respiratory cells were present only peripheral to the olfactory epithelium. In cell suspensions from adult rabbits, the numerical relationship between olfactory and respiratory cells was approximately 1:1 whereas in preparations from 1 to 2 month old rabbits there were fewer of the respiratory cells (Fig. 6). It is important for investigators of olfaction to know of the effects which may be introduced owing to the age of the experimental animals.

Studies on the dispersion of rabbit olfactory cells have been conducted to facilitate investigation of the molecular mechanisms of odor-sensing. The olfactory cell suspensions can now be subjected to density gradient centrifugation in order to separate the individual cell types. If achieved, this separation will facilitate detailed biochemical investigation of each cell type to determine its importance in the odor-sensing mechanisms.

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## REFERENCES

1. ALBERS, R. W., RODRIGUEZ DE LORES ARNAIZ, G., and DEROBERTIS, E., *Nat. Acad. Sc.*, 1965, **53**, 557.
2. ALTMAN, P. L., Blood and Other Body Fluids, (Biological Handbook) Washington, D.C., Federation of the American Society for Experimental Biology, 1961.
3. BENJAMIN, R. M., HALPERN, B. P., MOULTON, D. G., and MOZELL, N. N., *Ann. Rev. Psychol.*, 1965, **16**, 381.
4. BRACHET, J., and MIRSKY, A. E., (editors), The Cell, New York, Academic Press Inc., 1959, **I**, 1–816.



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5. COOPERSTEIN, S. J., and LAZAROW, A., *J. Biol. Chem.*, 1951, **189,** 665.
6. GORI, G. B., *Appl. Microbiol.*, 1964, **12,** 115.
7. GRAY, E. G., and WHITTAKER, V. P., *J. Anat.*, 1962, **96,** 79.
8. GWATKIN, R. B. L., and THOMSON, J. L., *Nature*, 1964, **201,** 1242.
9. HOGEBOOM, G. H., SCHNEIDER, W. C., and STRIEBICH, M. J., *J. Biol. Chem.*, 1952, **196,** 111.
10. HOSIE, R. JEANETTE A., *Biochem. J.*, 1965, **96,** 404.
11. JONES, F. N., and JONES, MARGARET H., *J. Psychol.*, 1953, **36,** 207.
12. LEGROS CLARK, W. E., *Proc. Roy. Soc. London, Series B*, 1957, **146,** 299.
13. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
14. MATEYKO, G. M., and KOPAC, M. J., *Ann. New York Acad. Sc.*, 1963, **105,** 183.
15. NEGUS, V., The Comparative Anatomy and Physiology of the Nose and Paranasal Sinuses Edinburgh, E. S. Livingstone Ltd., 1958.
16. OTTOSON, D., *Pharmacol. Rev.*, 1963, **15,** 1.
17. SMITH, L., and CAMERINO, P. W., *Biochemistry*, 1963, **2,** 1432.
18. VARON, S. S., RAIBORN, C. W., JR., SETO, T., and POMERAT, C. M., *Z. Zellforsch. u. Mikr. Anat.*, 1963, **59,** 35.
19. WALDER, A. I., and LUNSETH, J. B., *Proc. Soc. Exp. Biol. and Med.*, 1963, **112,** 494.
20. WHIPPLE, H. E., (editor), *Ann. New York Acad. Sc.*, 1964, **116.**
21. WHITELOCK, O. V. ST., *Ann. New York Acad. Sc.*, 1959, **81,** 219.
22. ZOTTERMAN, Y., (editor), Proceedings of the First International Symposium on Olfaction and Taste, New York, The Macmillan Company, 1963.



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