ADVANCES IN ORAL BIOLOGY

Advances in Oral Biology

Edited by

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Preface

A high incidence of dental disease involving irreversible destruction of the teeth and their supporting structures is characteristic of all civilized communities. Yet preventive measures are still only in the experimental stage because of inadequate knowledge of the many factors involved in the maintenance of oral health and the initiation of disease. Progress is further hampered because the information required to initiate fundamental research is scattered throughout the literature of a wide range of subjects, apparently unrelated except in the context of oral diseases. It also happens that those interested in this field are distributed in small groups widely separately geographically. Thus communication between groups and wide dissemination of essential knowledge proceeds all too slowly.

The primary object of *Advances in Oral Biology* is to facilitate communication between dental scientists by providing critical surveys of the state of knowledge in selected areas of biology that bear upon growth, development, and maintenance of normal function of oral tissues on the one hand, and on the other, departures from this norm that eventually become recognized as disease. The value of this broad approach is well illustrated by the contributions appearing in this first volume, wherein the authors show the extent to which a multidisciplined approach has led to the acquisition of new information about the structure, chemical composition, and function of oral tissues. While the majority of authors will have the viewpoint of basic science, it may be expected that later volumes in the series will include some contributions with the clinical approach, dealing with the application of basic research described and disseminated in earlier volumes.

Advances in Oral Biology will attempt to define current problems in terms familiar to all scientists. I hope then that the series will also appeal to many at present not engaged in this field; for I believe that the inability of the majority of the dental profession to communicate with those working in the basic sciences has prevented many of the latter from seeing that the study of oral biology in relation to oral health and disease offers great opportunities for fundamental studies in their own disciplines.

October, 1963 PETER H. STAPLE

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Secretion of Saliva

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I. Introduction

The salivary secretions play a dominant role in determining the nature of the external environment of the oral structures and hence are of importance in maintenance of the structural and functional integrity, i.e., health, of these structures. The secretions are of importance, as well, in the processes of mastication, deglutition, chemical digestion, and, occasionally, in regulation of the fluid and electrolyte economy of the whole body. Saliva is also involved in mediation of taste. In some instances, the saliva may serve as a route for excretion.

The variety and diversity of functions of the salivary fluids are not unique; other digestive fluids possess similar or comparable functions. Even the very processes by which the saliva is formed, and the morphological integrity of the salivary tissues maintained, are probably shared in large part by digestive glands generally. Thus, from a general point of view, secretion of saliva may be treated as a model system for the general processes of digestive gland activity, wherever these occur. For this, salivary secretion possesses the advantage, from an investigative point of view, that its products and, frequently, also the tissues from which these are derived, are relatively easy to obtain, especially from laboratory animals. For modern physiology whether basically or clinically oriented, it is from this general point of view that salivary secretion is of greatest interest and that its study is likely to be of greatest fruitfulness.

In this review, topics have been arbitrarily selected mainly with regard to their underlying relevance to general processes in digestive gland function. Experimental methods have not been emphasized, except with regard to collection of salivary secretions from human beings. This is justified because the ease with which samples of total mixed saliva may be obtained from human subjects is deceptive. Total mixed saliva is a mixture which may be quite inconstant in composition because contributions by component secretions vary, with degree of stimulation particularly. For most studies, collection of separate secretions is highly desirable. Collection methods for laboratory animals will not be specifically discussed. With laboratory animals the temptation to collect a mixed fluid has not been as strong, and experimental procedures are generally well outlined in the papers to which reference has been made here. The selection of topics for this review has excluded from consideration many aspects of secretion, including pathological aspects, of admitted importance. These remain for future discussion. For the present, it is fortunate that a number of good reviews, of special topics, and compendia of literature have appeared in recent years, and these may be cited as supplements. Included are reviews or monographs by Babkin (1960), Emmelin (1952, 1961), Lundberg (1958), Rauch (1959), Afonsky (1961), Jenkins (1960), Junqueira and Hirsch (1956), Office of Naval Research (1960), Burgen and Emmelin (1961), Kerr (1961), Ostlund (1953), and by a number of workers in the annals of a conference on the Metabolism of Oral Tissues, edited by Person (1960).

II. Collection of Saliva in Man

Investigation of salivary secretion in man is limited by the obvious

difficulty in obtaining gland tissue and in modifying internally to any drastic extent the conditions under which the glands secrete. Nonetheless, the secretions themselves are readily obtainable from human subjects and relationships between salivary composition and many systemic or local oral conditions are susceptible to investigation. Methods of obtaining the salivary secretions, therefore, merit consideration.

Total mixed saliva is readily obtained from the oral cavity by simple methods. For collection of this fluid from subjects in the resting, waking state, the simple expedient of permitting saliva to accumulate in the floor of the mouth for a timed period, at the termination of which the fluid is expectorated into a collecting vessel, is probably best, since even the use of a saliva ejector seems to increase appreciably the extent of reflex stimulation (L. H. Schneyer, 1956a). Collection of secretions from individual glands is also feasible either by direct cannulation or, more conveniently, by the use of special mechanical devices. This is most successfully accomplished, from parotid gland, using the small chamber, held in place over the opening of Stenson's duct by a vacuum ring, first described by Carlson and Crittenden in 1910 and later popularized by Lashley (1916). Shannon *et al.* (1962) have recently described a modified parotid cap and have briefly reviewed the history of this device.

Collection of pure secretions, separately, from submaxillary and sublingual glands has posed difficulties, some of which are not yet fully solved. Lashley (1916) and Krasnogorski (1931) obtained separate submaxillary secretions by use of metal appliances similar in principle to that of the parotid cap. More recently, a method which permits separate collection of submaxillary and sublingual secretions has been reported by L. H. Schneyer (1955). This method involves a modification of a device described in 1912 by Pickerill for the collection of mixed submaxillary-sublingual saliva. The method described by Schneyer involves construction of a separate acrylic appliance for each subject, since the appliance must fit accurately into the floor of the mouth. The tissue-bearing surface of the saliva "separator" is shown in Fig. 1. Since the appearance of this method, a number of modifications have been reported. Parkins and Williams (1959) have prepared saliva "separators" as aluminum castings; this has the advantage of permitting autoclaving for sterilization. Henriques and Chauncey (1961) have built a bite plane into the appliance to permit chewing movements during collection. Block and Brottman (1962) have reported a modification, to permit collection of submaxillary secretion only, which may reduce somewhat the tedium of preparing individual appliances. These workers use a universally applicable shell, which can be individually adapted with rubber-base impression material.

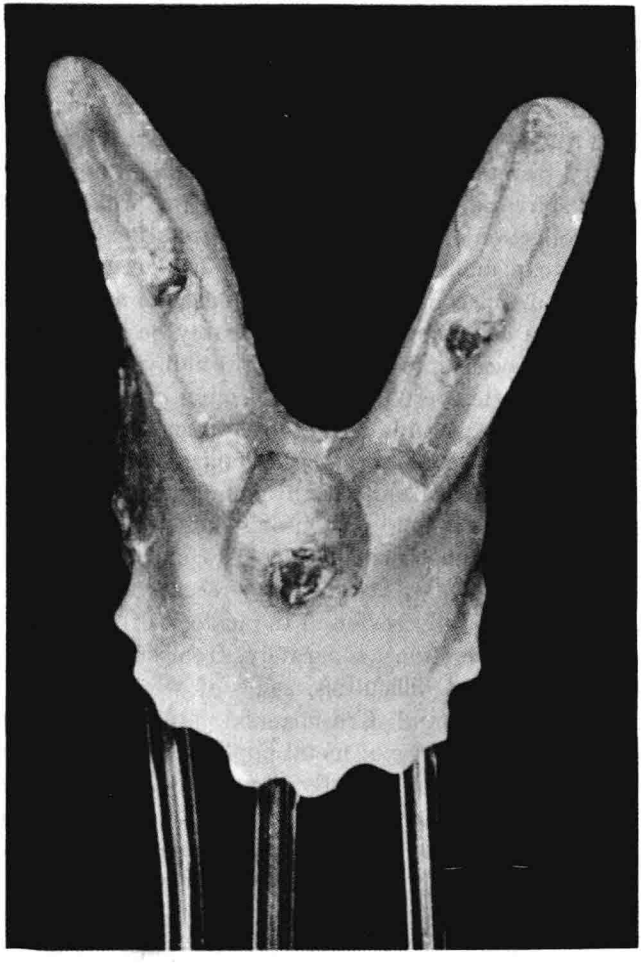

FIG. 1. Inferior surface of an acrylic appliance used to collect separate submaxillary and sublingual salivas from human subjects. Note lateral sublingual and medial submaxillary chambers and exit of collection tubes. Reproduced from L. H. Schneyer (1955).

Although discussion of the rate of flow and composition of the salivary secretions from human beings will not be attempted here, brief reference to work on these aspects should be convincing of the need for collection of the individual secretions for most types of investigation. Thus, while virtually all of the total mixed saliva, in the resting state at least, appears to come from the major salivary gland pairs (L. H. Schneyer, 1956a), these not only secrete at divergent rates (L. H. Schneyer and Levin,

1955a) but the contribution of each to the total secretion depends on intensity of the stimulus to secretion (L. H. Schneyer and Levin, 1955b). Thus, even for a range of flow rates within which the concentration of a substance in each component secretion does not change, the concentration in the mixed saliva will vary in accord with the proportionate contributions of the separate secretions at each flow rate, if the concentration of the substance differs in the various individual secretions (L. H. Schneyer, 1956b).

While it would be expected that the introduction of mechanical collection devices into the oral cavity could result in some stimulatory effect, this does not seem to occur to any appreciable extent (Lashley, 1916; L. H. Schneyer, 1955). Thus, methods are available, which are applicable in either resting or stimulated conditions, for separate collection of the secretions from the three pairs of major salivary glands of man.

III. Postnatal Development and Maintenance of Salivary Gland Structure

Salivary glands are extremely labile structures morphologically. In the neonatal, as well as in the fully developed organism, the morphological state of the glands may be readily modified by hormonal or nervous influences. The influence of the nervous system in development and maintenance of the glands is at present receiving a great deal of attention. It is interesting that, in turn, the salivary glands themselves appear to exert a growth-modifying effect on the nervous system. It is with aspects of postnatal development and maintenance of the salivary glands as influenced by the autonomic nervous system, or autono-mimetic drugs, that this discussion will be primarily concerned.

The salivary glands are not fully developed in the rat at birth, and there is some evidence that this is true of other species as well. Specifically, Jacoby and Leeson (1959) have shown that definitive acini and granular tubules are not present in the submaxillary gland of rat at birth, although well-developed striated ducts are already apparent; furthermore, definitive acini do not become conspicuous during the first few weeks of neonatal development, and granular tubules do not become visible until the sixth week. It has been shown recently that acinar development in the rat parotid gland is also not complete at birth (C. A. Schneyer and L. H. Schneyer, 1961b). Even though acini are not developed at birth in either the submaxillary or parotid gland, a copious secretion of definable composition has been obtained from the oral cavity

of neonatal rats 1 to 4 days of age (C. A. Schneyer and L. H. Schneyer, 1961b), and secretion directly from salivary ducts has been observed in animals ranging in age from 2 to 10 days.

Once developed, the integrity of the gland appears to be dependent upon a number of factors. Of these, the hormonal factors will not be discussed at this time. Possible regulatory effects of the nervous system, however, will be considered in some detail.

During acute stimulation of the salivary glands by way of the autonomic innervation, or by autono-mimetic drugs, transitory modification of morphology is effected. The specific cytological changes appear to be related to the kind of autonomic stimulation involved. Thus, Rawlinson (1935) reported changes in the cells of the striated ducts in the submaxillary gland of the cat, following either parasympathetic or sympathetic stimulation. The alveoli, or acini, on the other hand, while markedly affected by parasympathetic stimulation, were morphologically relatively insensitive to acute stimulation by epinephrine or the sympathetic nerve supply (Rawlinson, 1933).

While the results of acute stimulation of the sympathetic nerve supply may not reveal an important regulatory influence for this branch, recent work involving chronic stimulation clearly suggests a role of the sympathetic nervous system in regulation of gland size and morphology. Wells *et al.* (1959) and Wells and Munson (1960) have shown that repeated cutting of the incisor teeth, in rat, will result in enlargement of the submaxillary gland. This enlargement has been shown by Wells, in a well-designed series of experiments, to be the result of an excessive stimulation of the glands that is mediated through the sympathetic nervous system (Wells, 1960). This enlargement depends upon the continued stimulus of repeated cutting of the incisors, and cessation of this stimulation causes reversibility of the gland enlargement. Hypertrophy of acinar elements is reported to be the cause of the gland enlargement.

More recently, Selye *et al.* (1961b) found that administration of very large (pharmacological) doses of the dimethyl analog of epinephrine, isoproterenol, causes a marked enlargement of the salivary glands in rat. These workers considered the enlargement to be a true growth response to the drug and indicated that the enlargement could be attributed more to hyperplasia than to hypertrophy. Seifert (1962) has reported similar effects. On the other hand, Brown-Grant (1961), using mice, and C. A. Schneyer (1962), using rats, although confirming the enlarging effects brought about by isoproterenol, find that this is primarily the result of marked increase in size of individual cells (acinar cells) and, further, are of the opinion that this enlargement is the direct result of stimulation of the cells to excessive activity. C. A. Schneyer (1962) also observed

mitosis in early stages but believes that only a small fraction of the enlargement is the result of hyperplasia. Support for this view derives from the observation that the enlargement is nearly completely reversible upon withdrawal of the drug. The heart, which also responds to sympathetic stimulation by increased activity, similarly enlarges greatly, and this enlargement is also, to a large extent, reversible within 14 days (C. A. Schneyer, 1962). These effects are shown by the data in Table I. By 30 days after withdrawal, the enlargement of the heart from isoproterenol is nearly completely reversed. The enlargement of both salivary glands and heart, brought about by isoproterenol (like submaxillary gland enlargement following cutting of the incisors), is only maintained

TABLE I

SALIVARY GLAND AND HEART VENTRICLE SIZE AFTER CHRONIC ISOPROTERENOL (ISO) TREATMENT[a]

Treatment	Organ size (mg)			
	Submaxillary	Parotid	Sublingual	Ventricles
None	204 ± 5 (18)	216 ± 11 (20)	37.2 ± 1.1 (15)	640 ± 20 (13)
ISO: 6–8 mg daily for 6–8 days	440 ± 38 (7)	741 ± 40 (7)	46.2 ± 2.6 (7)	825 (4)
ISO: 8–12 mg daily for 10–14 days	642 ± 37 (12)	1136 ± 44 (12)	46.0 ± 2.2 (12)	992 ± 56 (12)
ISO: 12 mg daily for 14 days; then no ISO for 10–14 days	257 ± 16 (8)	373 ± 35 (8)	35.7 ± .8 (8)	788 ± 68 (5)
% return[b] to normal size	88	83	100	58

NOTE: Values are means ± SE for one gland of each pair but for both ventricles; figures in parentheses are number of animals. For enlargement series, measurements were made 36–48 hours after last ISO injection.

[a] Reproduced from C. A. Schneyer (1962).

[b] Return calculated for animals given 12 mg daily for 14 days;

$$\% \text{ return} = \frac{(\text{size with ISO}) - (\text{Size after ISO withdrawal})}{(\text{size with ISO}) - (\text{original size})} \times 100$$

in the presence of the stimulating agent, although after greatly prolonged stimulation by isoproterenol (about 45 days), at least in the case of salivary glands, atrophy occurs (Selye *et al.*, 1961a). Function of the enlarged salivary glands (Brown-Grant, 1961; C. A. Schneyer, 1962) and heart (Beznak, 1962) is normal in many respects; the enlarged salivary glands have many biochemical and physiological characteristics of active glands (C. A. Schneyer, 1962).