Nerve growth factor and epidermal growth factor are found in appreciable amounts in the male mouse submaxillary gland. The appearance of both growth factor activities in mouse saliva has also been reported. This study was concerned with the characterization of the molecular forms of the two growth factors in saliva. No evidence of a new form of nerve growth factor in saliva was obtained. Instead essentially all of the nerve growth factor activity isolated from the saliva of epinephrine-stimulated mice was identified as the 7 S nerve growth factor complex previously isolated from extracts of the submaxillary gland. Saliva from unstimulated mice contained the same complex and a small amount of its biologically active subunit β nerve growth factor. A one-step procedure for the isolation of 7 S nerve growth factor was devised. The high molecular weight epidermal growth factor complex was also isolated from saliva. The only differences between 7 S nerve growth factor obtained from saliva and from the submaxillary gland were in the relative proportions of the various γ subunits and the appearance of a greater proportion of the β nerve growth factor chains which lacked the NH₂-terminal octapeptide sequence. The release of this octapeptide sequence occurred during secretion. The finding that both 7 S nerve growth factor and high molecular weight epidermal growth factor activity are secreted into saliva emphasizes the physiological significance of these two protein complexes.

Nerve growth factor and epidermal growth factor activities are found in relatively large quantities in the submaxillary gland of the adult male mouse. Why these growth factors should be concentrated in this particular gland is not yet understood. As one approach to this problem, a number of investigators have recently examined the submaxillary gland secretions for NGF⁴ and EGF. Angeletti et al. (1967) detected low levels (0.02% of that in gland extract) of biologically active NGF in pilocarpine-elicited saliva of the mouse, and Wallace et al. (1977) showed that NGF is secreted into saliva during α-adrenergic stimulation (Wallace et al., 1977). Similarly, EGF has been detected by radioimmunoassay in the saliva following α-adrenergic stimulation (Byyny et al., 1974). One important question which remains is the form in which NGF and EGF appear in saliva.

In the gland itself, the NGF activity is present in a protein, 7 S NGF, which has a molecular weight of 130,000 and contains three different types of subunits (Varon et al., 1977; Smith et al., 1968). Only one of these subunits, βNGF, a basic protein composed of two identical 13,250 molecular weight chains, has NGF activity (Smith et al., 1968). The other two subunits are the γ subunit, an arginine esteropetidase of molecular weight 26,000, which may be involved in the processing of βNGF from a precursor (Berger and Shooter, 1977, 1978), and the α subunit, an acidic group of proteins of molecular weight 26,500. The βNGF subunit can be purified directly from the submaxillary gland extract at mild acid or alkaline pH where γ S NGF is unstable. During its isolation under these conditions, βNGF is subject to limited proteolysis at its COOH terminus (Angeletti et al., 1973; Moore et al., 1974) and near its NH₂ terminus (Angeletti et al., 1973); the products are collectively called 2.5 S NGF (Bocchini and Angeletti, 1969; Angeletti et al., 1971). EGF has also been isolated from extracts of the gland and is associated with an arginine esteropetidase called RGF-binding protein (EGF-BP, molecular weight 26,000) in a high molecular weight complex, HMW-EGF (Savage et al., 1972; Taylor et al., 1970). It should be noted that other forms of mouse NGF have been described. NGF secreted by cultured mouse L cells contains biologically active 2.5 S NGF in a molecular weight species of 160,000 (Pantazis et al., 1977). The NGF in mouse saliva has recently been shown to be present in both high and low molecular weight forms (Murphy et al., 1977). The high molecular weight form, reported to be different from 7 S NGF (Murphy et al., 1977), gave rise to the low molecular weight form which was thought to be identical in size with a single peptide chain of βNGF.

It has been confirmed, as described here, that high levels of biologically active NGF are present in epinephrine-elicited saliva from the adult male mouse. However, we have found no evidence either of a new high molecular weight or of a low molecular weight NGF species in mouse saliva. On the contrary, the major species present is 7 S NGF, and its secretion in saliva emphasizes the physiological significance of the complex which contains NGF. Similarly, EGF is present in saliva in its high molecular weight form, again emphasizing the physiological significance of the high molecular weight complex.
MATERIALS AND METHODS

Animals—Swiss Webster male mice 100 to 120 days old (35 to 45 g) were obtained from Simonsen Laboratories and maintained on laboratory animal chow and water, ad libitum.

Chemicals—Epinephrine (1 mg/ml) was obtained from Parke-Davis Co., sodium pentobarbital (60 mg/ml) from Diamond Laboratories, N-benzoyl-D-arginine-p-nitroanilide hydrochloride from Aldrich, amphotericin (pH 3.5 in 100 mM) from 1-KR-Prodaktor AR, bis-Tris from General Biochemicals, Tes from Calbiochem, NAD (carrying free 1.4 to 2.2 x 10^6 Ci/ml) from New England Nuclear, Sephadex G-100 and blue dextran 2000 from Pharmacia, DE52 and CM52 (microgranular preswollen) from Whatman, ultrapure urea from Schwarz/Mann, and F-12 medium with 5% fetal calf serum from Gibco. All buffers and salts were reagent grade.

Other Proteins—Hexokinase was obtained from Nutritional Biochemical Co, and ovalbumin (twice crystallized) from Sigma, and soybean trypsin inhibitor from Worthington Biochemical Co.

Isolation of HMW-EGF and of 7 S NGF and Its Subunits from Saliva—Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). The epinephrine-induced saliva was collected by the procedure of Wallace and Partlow (1976), frozen immediately over dry ice, and stored at −20°C until analyzed. An average of 180 ± 27 μl of saliva was obtained/mouse; its pH was 8.0 ± 0.25. These data are based on measuring the volume and pH of four pools of saliva, each collected from 15 to 25 mice. The NGF content of the saliva was 90 ± 16 ng/mouse (expressed in terms of mouse saliva by gel filtration on Sephadex G-100 (2.5 x 92 cm) equilibrated with the same buffer. Flow rate was 30 ml/h and fraction volume was 2 ml. Major protein peaks were pooled and concentrated by vacuum dialysis prior to further purification and/or characterization. The salivary 7 S NGF was identified in peak II of the G-100 elution profile (Fig. 1) by biological assay with precipitation with antibody to βNGF and the HMW-EGF protein complex in peak III by isoelectric focusing.

The α, γ, and βNGF subunits of 7 S NGF were isolated by the procedure of Smith et al. (1968) from peak II HMW-EGF complex purified to peak III by the method of Taylor et al. (1970). HMW-EGF, 7 S NGF, and the α and γ subunits were stored at −20°C in 0.05 M Tris-HCl buffer, pH 7.4, and βNGF at −20°C in 0.025 M acetic acid.

Protein Determinations—Protein concentrations were estimated spectrophotometrically using the known extinction coefficients (ε1%1 cm) of 7 S NGF (15.4), βNGF (16.0), γ subunits (15.6), α subunits (14.8), and HMW-EGF (19.4) (Servier and Shooter, 1976; Taylor et al., 1975).

Electrophoresis and Isoelectric Focusing in Acrylamide Gels—Isoelectric focusing in 10% acrylamide gels using a pH 3.5 to 10 Tris/Tes system, pH 7.05, followed the procedure of Server and Shooter (1976). Following electrophoresis or isoelectric focusing, gels were fixed in 20% trichloroacetic acid and scanned at 280 nm in a Gilford 2000 spectrophotometer. Gels were stained using 1% acid-fast green in methanol/acetic acid/H₂O (45:10:45 by volume) for 2 h at 37°C and destained in the same solvent or stained in Malzk and Berne rapid stain (Malik and Berrie, 1972) for 24 h and scanned at 580 nm.

Electrophoresis in 12.5% acrylamide sodium dodecyl sulfate-urea gels followed the method of Swank and Munkres (1971) and was continued for 60 mA h. The gels were stained as described above using acid-fast green.

Enzyme Assay—Arginine esterase activity of several γ subunit preparations from submaxillary gland homogenate and saliva was measured using the synthetic substrate, BAPNA. Assays were performed in 1-cm pathlength cuvettes. BAPNA in MeSO (0.100 M) was added to 0.05 M Tris-HCl buffer, pH 7.4, 1 μl of enzyme sample, and substrate concentrations ranging from 0.02 to 1 ml. The solution was rapidly mixed, 5 μg of γ subunit or 12.5 μg of 7 S NGF (approximately 5 μg of γ subunit) added, and mixed again. Hydrolysis of BAPNA was monitored at 410 nm on a model 25 Beckman spectrophotometer with a strip-chart potentiometric recorder. A double-reciprocal plot was used to determine Vmax and Km.

When 7 S NGF was assayed directly for γ subunit activity, 0.001 M EDTA was added to the cuvette and incubated for 10 min prior to the addition of BAPNA to facilitate full dissociation of the high molecular weight complex (Pattison and Dunn, 1976).

RESULTS

Form of NGF in Epinephrine-elicited Saliva—The analysis of mouse saliva by gel filtration on Sephadex G-100 is shown in Fig. 1. When assayed for NGF by immunoprecipitation at pH 4.0, all of the NGF originally present in the saliva appeared in peak II (Table I). Traces only of NGF appeared in peaks III and IV and none in peak I. The presence of essentially all NGF in peak II suggested that saliva NGF exists in a high molecular weight form.

Peak II from the Sephadex G-100 gel filtration of saliva was concentrated and compared to submaxillary gland 7 S NGF.
by both electrophoresis (Fig. 2) and isoelectric focusing (Fig. 3). At pH 7.05, 7 S NGF from the submaxillary gland does not dissociate and migrates as a single band (Fig. 2A). The protein in peak II showed only a single band which co-migrated with 7 S NGF (Fig. 2, B and C). In the analysis by isoelectric focusing, the protein in peak II showed the typical pattern of the submaxillary gland 7 S NGF subunits (Fig. 3, A and B) with only one minor difference. The appearance of three

<table>
<thead>
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<th>TABLE I</th>
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<td>NGF content of mouse saliva and of fractions derived from saliva</td>
</tr>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>1. Whole saliva</td>
</tr>
<tr>
<td>2. Dialyzed saliva</td>
</tr>
<tr>
<td>3. Peak II from G-100 column</td>
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</table>

*Preparation of the fractions is described under "Materials and Methods." *Determined by immunoprecipitation using anti-NGF antiserum and the data expressed as milligrams of βNGF.

Fig. 2. Comparison of the electrophoretic analysis of peak II with submaxillary gland 7 S NGF. Analysis was carried out by electrophoresis in 12% acrylamide gels in the bis-Tris/Tes system, pH 7.05, as described under "Materials and Methods." Gels were stained with Malik and Berrie (1972) fast stain and scanned at 580 nm. A, 100 µg of protein from peak II; B, 100 µg of submaxillary gland 7 S NGF; C, 50 µg each of protein from peak II and submaxillary gland 7 S NGF.

Fig. 3 (left). Comparison of the isoelectric focusing analysis of peak II with submaxillary gland 7 S NGF. Isoelectric focusing was in 10% acrylamide gels in a pH 3.5 to 10 gradient. Gels were stained with 1% acid-fast green as described under "Materials and Methods." A, 100 µg of protein from peak II; B, 100 µg of submaxillary gland 7 S NGF; C, 50 µg each of protein from peak II and submaxillary gland 7 S NGF.

FIG. 3 (left). Comparison of the isoelectric focusing analysis of peak II with submaxillary gland 7 S NGF. Isoelectric focusing was in 10% acrylamide gels in a pH 3.5 to 10 gradient. Gels were stained with 1% acid-fast green as described under "Materials and Methods." A, 20 µg of salivary α; B, 20 µg of submaxillary α; C, 20 µg of salivary γ; D, 20 µg of submaxillary γ; E, 10 µg each of salivary and submaxillary γ; F, 20 µg of salivary βNGF; G, 20 µg of a mixture of submaxillary gland βNGF, monodes-Arg18-βNGF, and bisdes-Arg18-βNGF; H, 10 µg each of salivary and submaxillary gland βNGF.

Fig. 4 (right). Isoelectric focusing analysis of α, γ, and βNGF subunits of salivary and submaxillary gland 7 S NGF. Isoelectric focusing was performed in 10% acrylamide gels in a pH 3.5 to 10 gradient. Gels were stained with 1% acid-fast green as described under "Materials and Methods." A, 20 µg of salivary α; B, 20 µg of submaxillary α; C, 20 µg of salivary γ; D, 20 µg of submaxillary γ; E, 10 µg each of salivary and submaxillary gland γ; F, 20 µg of salivary βNGF; G, 20 µg of a mixture of submaxillary gland βNGF, monodes-Arg18-βNGF, and bisdes-Arg18-βNGF; H, 10 µg each of salivary and submaxillary gland βNGF.


**Table II**  
Relative ratios of subunit species and of subunits within 7 S NGF

<table>
<thead>
<tr>
<th>Subunit source</th>
<th>α/α′/α″</th>
<th>γ/γ′/γ″</th>
<th>βNGF:monodes-Arg&lt;sup&gt;112&lt;/sup&gt;/βNGF des(1-8):βNGF chains</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Epinephrine-elicited saliva</td>
<td>±2 ±2 ±4</td>
<td>±3 ±1 ±3</td>
<td>+9 +9 +9</td>
</tr>
</tbody>
</table>

*Mean ± S.D., n = three preparations.

This includes both γ<sup>′</sup> and γ″.

**Table III**  
Comparison of enzymatic activities of γ subunit isolated from submaxillary gland and from saliva

<table>
<thead>
<tr>
<th>Source</th>
<th>K&lt;sub&gt;e&lt;/sub&gt;</th>
<th>V&lt;sub&gt;max&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Submaxillary gland extract</td>
<td>92 ± 2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>2. Epinephrine-elicited saliva</td>
<td>95 ± 8</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>3. Saliva (7 S NGF)</td>
<td>3.0 ± 1.3</td>
<td></td>
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*Mean ± S.D., n = three preparations.

7 S NGF rather than the γ subunit was used here.

Bioassay of Salivary NGF—The biological activity of βNGF isolated from saliva or in whole saliva was determined by the single cell bioassay (Fig. 6). The βNGF concentrations required for half-maximal response of neurite outgrowth were identical for the saliva and submaxillary gland NGF.

Effect of Collection Procedure on Form of NGF in Saliva—The finding that all NGF is in a high molecular weight form in epinephrine-elicited saliva conflicts with the recent results of Murphy et al. (1977) who have suggested that mouse saliva contains both a new high molecular weight form of NGF and the single chain of βNGF. One factor which may

concentration was raised to 0.4 M NaCl, and the βNGF subunit eluted following a change of pH to 9.0 (Fig. 4). A typical yield was 2.5 mg, each, of α and γ subunits and 1.3 mg of βNGF from 10 mg of 7 S NGF.

The isolated α subunits from salivary 7 S NGF were identical in isoelectric points (Fig. 4, A and B) and in the distribution of protein between the various α species (Table II) to the submaxillary gland α subunits. Three of the four saliva γ subunits focused at the same pH as the γ subunits from the submaxillary gland (Fig. 4, C to E) while the fourth, designated γ″, focused between the γ′ and γ″ subunits. In addition, there were significant differences in the proportions of the γ subunits isolated from saliva and the submaxillary gland tissue (Table II). The saliva subunits contained more γ′, but less γ″, the latter being equally divided between the γ′ and γ″ subunits. In spite of these differences, the enzymatic properties of the saliva γ subunits were the same as those of the submaxillary gland γ subunits (Table III).

The isolated saliva βNGF had the same isoelectric point as submaxillary gland βNGF and, like the latter, contained approximately 10% of monodes-Arg<sup>112</sup>βNGF (Fig. 4F and Table II). The first 8 NH<sub>2</sub>-terminal amino acid residues of βNGF can be released as an octapeptide sequence by a specific endonuclease present in the submaxillary gland (Mobley et al., 1974).<sup>8</sup> In typical gland βNGF preparations, approximately 10% of the peptide chains lack the NH<sub>2</sub>-terminal octapeptide sequence as a result of the activity of this enzyme. In the saliva βNGF, significantly more of this specific proteolysis has occurred and approximately 30% of the βNGF chains lack the NH<sub>2</sub>-terminal octapeptide sequence.

Immunoprecipitation of Salivary and Submaxillary Gland βNGF—Antiserum prepared against βNGF from submaxillary gland quantitatively precipitated βNGF isolated from saliva. Two samples each of submaxillary gland βNGF and of salivary βNGF were tested against the antiserum (Fig. 5). All four samples have identical equivalence points, suggesting that βNGF from saliva has antigenic properties identical with those of submaxillary gland βNGF.

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<sup>8</sup>W. H. Wilson and E. M. Shooter, manuscript in preparation.
account for these differences is the various methods used for collecting the mouse saliva.

In this study, the method of Wallace and Partlow (1976) was followed and the saliva was collected over an approximate 10 min period in capillary tubes placed with one end in the mouth. Although collection of epinephrine-elicited saliva by this procedure included contamination from the parotid and sublingual glands, no new modifications of NGF were observed, and as noted above all NGF was in the 7 S NGF form. Replication of the Murphy et al. (1977) procedure which involved cutting the gland ducts and collecting saliva over an approximate 45-min period from the pretrachael space with capillary tubes likewise produced saliva unavoidably contaminated with unidentified substances, including lymph and fluid from possibly damaged tissue. This saliva, collected in the absence of adrenergic stimulation, contained only about 20% of the amount of NGF in saliva collected orally under adrenergic stimulation. Unlike orally collected saliva, it had significant amounts of proteins which eluted in the void column of the Sephadex G-100 column. However, there were only minor differences between the two salivas in the proteins of the Sephadex G-100 column. However, there were only minor differences between the two salivas in the proteins which entered the acrylamide gel on isoelectric focusing (data not shown). Furthermore, the analysis of the proteins from the peak II region of the gel filtration eluate of the saliva collected from the pretrachael space showed all of the subunits of 7 S NGF. The main difference between the two salivas was the proportion of NGF observed in peak IV of the eluate. This reached as high as 10% of the total NGF applied to the column in some salivas collected from the pretrachael space. In contrast, saliva collected from the pretrachael space of adrenergically stimulated mice, where pharmacological stimulation reduced both collection time and contamination, was similar in its 7 S NGF level to orally collected saliva and contained no low molecular weight NGF.

*Isolation of HMW-EGF from Saliva—Peak III from the gel filtration on Sephadex G-100* (Fig. 1) was further fractionated by the procedure used to obtain HMW-EGF (Taylor et al., 1970). The final product had the same electrophoretic mobility as submaxillary gland HMW-EGF when analyzed by electrophoresis at neutral pH (Fig. 7, D to F). The two proteins were also compared by isoelectric focusing in a pH 3.5 to 10.0 gradient under conditions where HMW-EGF dissociates. The subunit pattern (EGF and EGF-BP) for the saliva protein was identical with that of submaxillary gland HMW-EGF both in terms of the isoelectric points of the component subunits and their distribution (Fig. 7, A to C).

The saliva HMW-EGF had the same elution volume as submaxillary gland HMW-EGF on gel filtration on Sephadex G-100, giving an apparent molecular weight for both proteins of 52,000.

**DISCUSSION**

The data presented here show that 7 S NGF is the major form of NGF in the saliva of epinephrine-stimulated mice. The fact that this high molecular weight complex is secreted into saliva, in a form which is essentially identical with that in the submaxillary gland itself, offers convincing evidence that 7 S NGF is a physiologically significant protein complex. The same holds true for the HMW-EGF complex. In particular, it seems unlikely that either complex is an artifact generated during the isolation procedure, as suggested by Murphy et al. (1977), because, first, the protein composition of homogenates of the submaxillary gland and of saliva is different and, second, only one fractionation step is required to isolate salivary 7 S NGF. Since the isolation of 7 S NGF from saliva avoids the use of ion exchange columns which may subtly alter the stoichiometry of the 7 S NGF complex, the salivary 7 S NGF is an ideal preparation for further detailed studies of its subunit composition.

The differences between saliva and submaxillary gland 7 S NGF are minor but are of interest. The finding of an increased proportion of the y' subunit in salivary gland 7 S NGF compared to submaxillary gland 7 S NGF suggests that if the y' and y' subunits arise by proteolytic modification of the y subunit, then less proteolysis occurs during their isolation from saliva than from homogenates of the submaxillary gland. This suggestion seems reasonable since the isolectric salivary 7 S NGF requires only one gel filtration step. The other difference between salivary and submaxillary gland 7 S NGF is the extent of cleavage of the NH2-terminal octapeptide from bNGF, being greater in saliva than in submaxillary gland homogenate. Since incubation of 7 S NGF in saliva does not lead to further release of the NH2-terminal octapeptide, the extra cleavage must take place during the secretion process. The mechanism by which this occurs as well as the physiological relevance of the release of the octapeptide remains unclear.

The recent results of Murphy et al. (1977) have suggested that mouse saliva contains both a new high molecular weight form of NGF and the single chain of bNGF. Two characteristics of their high molecular weight species led Murphy et al. (1977) to suggest it was different from 7 S NGF, namely its molecular weight and its stability at low concentrations. However, the difference in molecular weight between 7 S NGF and the species identified by Murphy et al. (1977) is not significant. They report a molecular weight of 114,000 for their salivary NGF species as estimated from gel filtration on Sephadex G-200 and of 89,000 for the submaxillary gland NGF species by the same technique. They considered that these two values were identical and that the differences fell within the error of the method. Although 7 S NGF has an actual molecular weight of 130,000, its apparent molecular weight determined by gel filtration on Sephadex G-100 is approximately 93,000.
The high molecular weight salivary NGF species studied by Murphy et al. (1977) is, therefore, indistinguishable from 7 S NGF in terms of its molecular weight.

With respect to the reported stability of the high molecular weight salivary NGF (Murphy et al., 1977), this is again entirely consistent with the known properties of 7 S NGF. The stability of 7 S NGF is altered dramatically by zinc ions (Pattison and Dunn, 1975). In the absence of zinc ion, the equilibrium constant for the dissociation of the $\gamma$ subunit from 7 S NGF is $10^{-7}$ M. The equilibrium constant decreases progressively with added zinc ion to reach a value of $10^{-12}$ M at zinc ion concentrations of $10^{-6}$ M or greater (Bothwell and Shooter, 1978). The stability of 7 S NGF in saliva is therefore a function of both its concentration and of the free zinc ion content of the saliva. The total zinc content of saliva is very high ($10^{-4}$ M, Footnote 4) and even if much of it is complexed with other constituents, the residual free zinc ion concentration is likely to be of the order of $10^{-7}$ M and, therefore, still sufficient to keep the equilibrium dissociation constant of 7 S NGF in the range of $10^{-10}$ to $10^{-11}$ M. It follows that 7 S NGF, which is present in epinephrine-elicited saliva at a concentration of approximately 3.4 mg/ml, will be completely stable. The results of the present study confirm this prediction. The stability of the high molecular weight form of NGF described by Murphy et al. (1977) is, therefore, also consistent with its being 7 S NGF.

Elution of NGF activity at the position of cytochrome c, following Sephadex G-75 chromatography of saliva, led Murphy et al. (1977) to suggest that the low molecular weight NGF present in saliva was the single chain of $\beta$NGF with a molecular weight of 13,250. Recent work has, however, shown that the NGF dimer does not dissociate to form significant amounts of monomer at any concentration down to $10^{-15}$ M and also that when $\beta$NGF is chromatographed on a column of Sephadex G-75, it elutes close to cytochrome c (Bothwell and Shooter, 1977). This suggests, therefore, that the low molecular weight species described by Murphy et al. (1977) is identical with $\beta$NGF.

The failure to detect low molecular weight $\beta$NGF in saliva collected after adrenergic stimulation suggests in turn that its appearance in unstimulated saliva is a result of a proteolytic degradation which decreases its affinity for the other subunits of 7 S NGF. The cleavage of the COOH-terminal arginine residues of $\beta$NGF has such an effect (Moore et al., 1974; Perez-Polo and Shooter, 1975;Mobley et al., 1976) and it has been noted that this cleavage occurs when $\beta$NGF is incubated in saliva. It is possible, therefore, that the prolonged collection time required to obtain sufficient saliva from unstimulated mice, together with the low concentration of 7 S NGF in this saliva, provides conditions suitable for the proteolytic release of the critical COOH-terminal residues. Finally, it is not clear from the data of Murphy et al. (1977) how much modified $\beta$NGF appears in saliva collected by their method. The quantitation of NGF by their radioimmunoassay leads to an underestimation of $\beta$NGF present as 7 S NGF because the anti-$\beta$NGF antiserum precipitates less 7 S NGF than $\beta$NGF at a given antiserum volume. This leads to a bias in the relative proportions of 7 S NGF and $\beta$NGF in favor of the latter.

Previous workers have demonstrated the presence of epidermal growth factor in epinephrine-elicited saliva (Taylor et al., 1970). The isolation of HMW-EGF from saliva is suggestive that this hormone also exists physiologically in a high molecular weight complex. Although the significance of its presence in saliva is poorly understood, its structural similarity to urogastrone (Gregory, 1975) and its effective inhibition of gastric acid secretion (Bower et al., 1975) may reveal gastrointestinal functions to be relevant to its physiological role. Similarly, in light of the extensive sympathetic innervation in the gastrointestinal system and the presence of large quantities of NGF in saliva, additional undisclosed roles for NGF may exist.

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