STUDIES IN SALIVARY GLANDS AND THEIR SECRETIONS IN HEALTH AND DISEASE

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SUMMARY

Some new methods and some established ones have been applied to studies of salivary glands in health and certain disease states.

A technique of recording the pattern of salivary flow has made it possible to collect saliva samples accurately at several different but constant flow rates. The effect of varying salivary flow rate on the parotid and submandibular concentrations of electrolytes, iodide, uric acid and also the activity of carbonic anhydrase has been demonstrated and the normal ranges defined. It is important to relate the concentration of some salivary constituents, e.g. iodide, to the plasma level.

Combined quantitative and radioisotopic methods have been used to determine basic values for the metabolism of iodine in salivary glands and saliva. Measurement of the salivary specific activity after a tracer dose has the advantage that it is independent of flow rate as the ratio of stable to radioiodine is constant at different flow rates. Normal ranges have been described for salivary iodide concentration, clearance, absolut quantities secreted in unit time and saliva/plasma ratios. The salivary iodide concentrating mechanism is normal in altered states of thyroid function and also in fibrocystic disease where previously high salivary iodide levels had been reported. In Sjögren's syndrome however low saliva/plasma ratios have been found and this suggests that the salivary iodide trap may be involved in this condition.

The chemical nature of the salivary iodine has been studied and found to be almost entirely in the inorganic form in health and in some thyroid disease states. In contrast the urinary iodine, normally inorganic, contains organic iodinated compounds in thyrotoxicosis and in dehalogenase deficiency. Some advantages of the salivary specific activity method over the urinary method for the indirect measurement of the plasma inorganic iodine are demonstrated.

Anions of the VIIth periodic group include iodide, bromide and pertechnetate. Pertechnetate, like iodide, is concentrated in saliva. Simultaneous administration of isotopes ^{132}I and $^{99m}TcO_4$ allow direct comparison of salivary gland concentrating ability to be made on the same saliva sample thus eliminating the variable of flow rate. The isotope $^{99m}TcO_4$ has many advantages over isotopes of iodine as a clinical tracer and its use for radioisotopic visualisation of the salivary glands has been demonstrated for the first time.

The criteria for diagnosis of oral and salivary gland involvement in Sjögren's syndrome have been examined. The comparative value of tests of salivary gland function have been assessed in 30 patients with a clinical diagnosis of Sjögren's syndrome. The advantages of the new technique of hydrostatic sialography are demonstrated. Good correlation was shown between sialographic appearances, salivary flo

rate measurements and clinical signs and symptoms. Labial gland biopsy shows a high occurrence of focal lymphocytic infiltration in patients with Sjögren's syndrome as compared with a control series. The onset of xerostomia and xerophthalmia showed no well defined relationship to the menopause. Both these components of the 'sicca syndrome' may arise together but often they commence and progress independently. While a high incidence of auto-immune thyroid disease is found in Sjögren's syndrome, an increased incidence of Sjögren's syndrome was not found in patients presenting clinically with auto-immune thyroid disease.

STUDIES IN SALIVARY GLANDS AND THEIR SECRETIONS IN HEALTH AND DISEASE

VOLUME I

PART II. STUDIES OF SOME CHEMICAL CONSTITUENTS OF SALIVA

PART III. THE SALIVARY GLAND IODINE CONCENTRATING MECHANISM IN HEALTH AND DISEASE

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PREFACE

The work upon which this thesis is based was carried out during the past four years in the University of Glasgow Department of Medicine, Western Infirmary; Department of Pathology, Royal Infirmary: and the Dental Hospital and School. Some of the data contained in this Thesis has already been published, accepted for publication or read at scientific meetings.

PUBLICATIONS

1. Estimation of the plasma inorganic iodine in man - a comparison of methods (1965).
   J. Lab. clin. Med. 65: 500.
   (with R.McG. Harden & W.W. Buchanan).

2. Quantitative studies of iodide excretion in saliva in euthyroid, hypothyroid and thyrotoxic patients (1965).
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3. * The influence of flow rate on the salivary iodine concentration in man. (1966).
   Arch. oral Biol. 11: 235.
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4. * The salivary iodine trap: relation between salivary iodine excretion and the plasma inorganic iodine concentration (1966).
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5. * Problems of interpretation in studies of salivary constituents (1966).
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6. * Carbonic anhydrase activity in saliva in man (1966).
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(with S. Papadopoulos, S. MacFarlane, R.McG. Harden and W.D. Alexander.)

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(Abstract) J. dent. Res. (in press).
International Association for Dental Research-British Division (April 1966).

* Those marked with an asterisk represent major contribution by the author of the Thesis.

ABBREVIATIONS AND TERMS USED

'Resting' Saliva	Refers to saliva collected under conditions of minimal stimulation.
Whole Saliva Mixed Saliva	These terms are used synonymously and refer to the total saliva pooled in the oral cavity.
Submandibular Submaxillary	These terms are used synonymously by different authors. In this text submandibular is used except when quoting the work of others.
Iodide	This is used in the strict chemical sense to denote iodide ion or electrolytes containing the iodide ion, and not to mean iodine in general.
Iodine	Iodine is used to denote the element in either organic or inorganic form.
VIIth periodic group	The elements iodine, technetium and bromine are members of this group. Iodide, pertechnetate and bromide are their respective anions. ^{132}I, $^{99m}TcO_4$ and ^{82}Br are the respective radioistopes used.
PII	Plasma inorganic iodine (normal range 0.08 - 0.60 µg/100 ml in West of Scotland).
Sicca complex or Sicca syndrome	These terms refer to the combined oral and ocular dryness in Sjögren's syndrome.
±	Standard error of the mean unless otherwise specified.

INTRODUCTION

This Thesis describes a study of the salivary glands and their secretions in health and disease. It is divided into four parts.

Part I is concerned with the description and validation of the methods used. Some methods were developed for use in the hospital out-patient clinic. New techniques for monitoring the pattern of salivary flow, hydrostatic sialography, radioisotopic scanning and salivary gland biopsy are described and the results in normal subjects are reported.

Part II includes a brief review of human saliva - its function and chemical composition. Examples are given of the problems encountered in the analysis of salivary constituents. The effects of varying salivary flow rates on salivary electrolytes and uric acid concentrations and on carbonic anhydrase activity are reported.

Part III describes physiological and biochemical studies of the salivary iodide concentration mechanism. Chemical and radioisotopic methods are combined to provide quantitative studies of salivary iodine metabolism. The chemical nature of the salivary iodine is examined and the relationship of the salivary iodide to flow rate and the plasma level is demonstrated. The concentrating ability of the salivary gland for other members of the VIIth periodic group is compared with iodine. The effect of potassium perchlorate on the salivary iodide trap is examined.

The salivary iodide concentrating mechanism is studied in such diseases as Sjögren's syndrome, fibrocystic disease and altered states of thyroid function. Lastly, the salivary and thyroid trapping mechanisms are compared in health and disease.

In Part IV the results of a study of 30 patients with Sjögren's syndrome are reported. All complained of xerostomia and their oral and salivary gland involvement was investigated by salivary flow measurements, sialography and oral biopsy. The diagnostic value of these three techniques are compared. Finally, in view of the increased incidence of auto-immune thyroid disease which has been reported in Sjögren's syndrome, the corollary, i.e. the incidence of Sjögren's syndrome in auto-immune thyroid disease has been investigated.

PART I

METHODS USED TO STUDY SALIVARY GLANDS AND THEIR SECRETIONS

PART I

INTRODUCTION

At the outset of this study it was apparent that for several aspects of salivary gland function there were no techniques suitable for routine use in a hospital out-patient clinic. It was therefore necessary to devise collection methods and techniques for the measurement of salivary flow rate pattern, radioisotopic scanning and salivary gland biopsy, which could be used in an out-patient department. In this way it has been possible to define the normal ranges of these parameters and the variations which occur in some disorders of salivary gland structure and function.

The conventional hand injection sialographic technique was found to be unsatisfactory for the investigation of patients with Sjögren's syndrome. A hydrostatic method giving a constant pressure during the filling phase was therefore developed. The methods which will be described may be sub-divided into those used to study salivary secretion and others used primarily for the study of the glands themselves. With the exception of the autoradiographic studies which were carried out in hamsters all the methods described here were used in man.

a) Methods used to study saliva

1) Collection of saliva.

2) Recording the pattern of salivary flow.

3) Radioisotopic.

4) Chemical Analysis.

b) Methods used to study salivary glands.

1) Clinical

2) Sialography

3) Scanning

4) Autoradiography.

5) Biopsy.

CHAPTER I

THE COLLECTION OF SALIVA

Salivary studies may be conducted on samples of mixed or total saliva or on separated secretions of the major salivary glands. The choice depends upon the nature of the problem being investigated. If saliva as part of the dental environment is being analysed mixed saliva is usually collected. As the individual gland contribution to mixed saliva varies in volume and composition under various conditions, where the estimation of a particular salivary constituent is being measured, accurate reproducible measurements can only be obtained on separated parotid, submandibular or sublingual saliva. In the majority of tests which will be described here parotid saliva was used but on occasions, submandibular or mixed saliva was required. Various collection devices were tried initially but the following methods for collection of parotid, submandibular and mixed saliva were found to be the most reliable and accurate.

PAROTID SALIVA
1) Modified Carlson-Crittenden cup
2) Combined catheter and suction cup (Kerr 1961).

SUBMANDIBULAR SALIVA
1) Polyethylene catheter (Kerr 1961).
2) Segregator appliance (Schneyer 1955).
3) Segregator appliance (Block & Brottman 1962).

MIXED SALIVA

The advantages, disadvantages and indications for the use of each method will now be considered.

PAROTID SALIVA

1) Modified Carlson-Crittenden cup (Figure I, 1). This basic two chambered device is often referred to as the Lashley cup (Lashley 1916) but as pointed out by Terry and Shannon (1965) was first described by Carlson and Crittenden in 1910. The inner chamber was placed over the parotid duct orifice and the cup maintained by air suction applied through the outer chamber. The source of air suction used was a conventional water pump attached to a water tap in the laboratory used for salivary studies. The pump had a pressure range of 0 - 760 mm Hg and the pressure applied was approximately 200-250 mm Hg. For collections carried out in other hospitals a rubber ball syringe was used to provide air suction for a portable saliva collection kit. The original Carlson-Crittenden cup was made of metal and no details of its size are given by the authors. Various modifications have since been described (Lashley 1916, Richter & Wada 1924; Krasnogorski 1931; Finesinger and Finesinger 1937; Gore 1938; Curby 1953; Kerr 1955; Tsaturov 1957; Miller 1960; Suhara et al 1959; Shannon et al 1962). The cup used here was made of nylon which was well tolerated by the oral mucosa. The inner chamber diameter of 10 mm. outer chamber diameter of 20 mm, and depth of 4 mm, resulted in

satisfactory retention. Deeper cups tried initially were discarded for two reasons, firstly because their bulk was uncomfortable for the subject and secondly they were liable to be dislodged by the adjacent teeth.

There are, however, two disadvantages of cup methods. Firstly, on occasions, movement of the cup occurs away from the position of the parotid duct orifice in some patients when intra-oral stimuli are being chewed or sucked. Secondly, if the air suction pressure is too great the buccal mucosa is sucked into the air suction tube and occludes it allowing movement of the cup away from the mucosa elsewhere. Both of these complications can result in air penetrating to the inner chamber and air bubbles are included with the saliva as it passes down the collecting tube. The inclusion of air will render inaccurate, attempts to measure flow rate using the drop counting photo-electric method which is described in Chapter 2. When this complication did occur it was necessary to adjust the air pressure or insert a tapered polyethylene catheter through the inner tube and into the duct orifice as described by Kerr (1961).

2) Combined Catheter and Suction Cup (Kerr 1961). In this method a suitable polyethylene catheter was selected to fit the parotid duct orifice. The polyethylene catheter was inserted through a tube to the inner chamber of the suction cup until it projected $\frac{1}{2}$ - 1 cm beyond the fitting surface of the cup (Figure I, 2). The catheter, with the cup attached, was then

inserted into the parotid duct and when satisfactorily positioned air pressure was applied through the outer chamber, as before, to maintain the appliance in position. Numerous catheters of varying sizes (Portex polyethylene diameters .5 - 1.5 mm) were kept sterilised for this purpose (Figure I, 3). The ends of the catheters were polished on cloth to avoid trauma to the duct lining. The disadvantages of this method are that it is time consuming and less convenient for the patient. Ascending infection can also occur and strict asepsis is essential. However, retention of the appliance is excellent and air inclusion never occurs. It was therefore, not used routinely but always when retention or air inclusion problems presented in a particular subject.

SUBMANDIBULAR SALIVA

1) Polyethylene Catheter (Kerr 1961).

This was the only satisfactory method of submandibular saliva collection in edentulous patients where intra-oral stimuli were employed. A tapered polyethylene catheter (as described above) was fitted for insertion 2 - 3 cms through the duct orifice into the submandibular duct. If necessary, the submandibular duct orifice was dilated first, using a lacrimal probe dilator. Great care is necessary in the preparation, selection and fitting of the polyethylene catheter. Various sizes (Figure I, 3) were prepared and the tapered ends of the catheters polished on cloth before insertion.

In some subjects submandibular catheterisation was not possible. Causes of failure were lingual angulation of lower incisor teeth, narrow duct orifices, and laxity and mobility of the surrounding soft tissues.

2) Segregator Appliance (Schneyer 1955).

Where a sufficient number of lower anterior teeth remained to provide retention the segregator method as described by Schneyer (1955) was used (Figure I, 4). This appliance was made especially for each patient, like a partial denture, in clear acrylic. An extension lingually from the denture formed a chamber which was placed over and around the submandibular duct orifices. This method is only as good as the peripheral seal around the chamber in the floor of the mouth. But, on the other hand, too great a downward pressure by the edges of the appliance will occlude the submandibular ducts as they lie in the floor of the mouth. A balance has therefore to be obtained and this requires careful adjustment. The efficiency of the peripheral seal should be tested using a simple dye such as Evans Blue.

3) Segregator Appliance (Block & Brottman 1962).

A modification of the Schneyer method has been described by Block and Brottman (1962) in which a preformed plastic cup (Figure I, 5) is placed over the submandibular duct orifice. To maintain it in position denture rubber base impression material is placed over, around and

between it and the lingual surfaces of the lower teeth. This appliance is quickly constructed at the chairside whereas the Schneyer method requires laboratory preparation. Similar precautions are necessary to obtain adequate peripheral seal. One disadvantage of this method is lack of retention which occurs occasionally when intra-oral stimuli are being used to promote changes in flow rate.

MIXED SALIVA.

This was most satisfactorily obtained by asking the patient to spit at a controlled rate of once per minute during the period of collection as described by Kerr (1961).

MEASUREMENT OF SALIVARY VOLUME

As the volume of saliva collected was often only 1-2 ml this was measured accurately by weighing the collection bottles or tubes before and after the collection. The maximum error involved in assuming that the specific gravity of salivary secretions was 1.000 is 1% (Kerr 1961).

CHAPTER 2

RECORDING THE PATTERN OF SALIVARY FLOW

The concentrations of many of the constituents of saliva vary with flow rate. Since the original work of Heidenhain (1883) the variations in concentration of salivary constituents with flow rate have been confirmed by many workers, Thaysen et al (1954) Hildes and Ferguson (1955), Bates (1958), Dawes and Jenkins (1964), Mason et al (1966a).

Previous workers have reported various methods of measuring salivary flow rate. Kerr (1961) emphasised that an automatic outflow recording method was desirable to measure sudden variations in secretion rate. In neither of the two methods he described, however, can the saliva be readily collected for subsequent analysis. Richter and Wada (1924) and Kutscher and co-workers (1964) have described a method whereby the volume of flow is measured along a calibrated tube, but the pattern of flow was not recorded. Earlier workers (Krasnogorski 1931 and Lashley 1916) measured the outflow of saliva using drop counters, but these had the disadvantage that saliva stuck to the electrodes causing corrosion. To date, therefore, no method described combines a record of the pattern of the salivary flow with collection of the saliva for analysis of its constituents.

In practice, therefore, when saliva is collected for analysis, the rate of flow is usually obtained by dividing the volume of saliva secreted in unit time by the duration of the collection period. The assumption is made that the flow rate is constant throughout this period. However, it is well recognised that the flow rate varies with many factors - swallowing, chewing, and the rate of application of the stimulus (Kerr 1961). For example, if 5 ml. of saliva are collected in 10 minutes then the average flow rate is 0.5 ml/min. If 2 ml. of this total volume are secreted in one minute and the remaining 3 ml. in the other 9 minutes, then the saliva collected is a mixture of saliva secreted at 2 ml/min and 0.3 ml/min. It was felt necessary, therefore, to have a method of monitoring continuously the rate of flow throughout each collection period. A photo-electric drop counting system was therefore designed whereby the pattern of salivary flow was recorded continuously when the saliva was collected for analysis of the constituents.

The Photo-electric Drop Counting Salivary Flowmeter.

As shown in Figure I, 6 the instrument is designed around three identical photoelectric detectors into which are inserted polyethylene cannulae conveying saliva from the patient's salivary ducts. Simultaneously collections can be made from 3 salivary glands. Each detector consists of a glass drip chamber (A) screwed to a perspex housing

(B) which contains a photo-transistor and a 2.2 volt lens-ended lamp so arranged that the beam of light falling on the photo-transistor is interrupted by the falling drop. The detectors are mounted on an angled metal plate (C) so that each detector is equi-distant from the patient's mouth. The stand (G) allows this plate to be positioned both vertically and horizontally to suit each patient. To ensure that the drip chamber is vertical a spirit level (H) is incorporated in the base of the plate. The drip chambers are easily interchangeable and can be accurately aligned by means of the perspex slide (D) and finally locked in position by screw clamp (E). The saliva is collected for analysis in bottles (F) situated under each drip chamber.

The circuit diagram of the instrument is shown in Figure I, 7. The phototransistors TR_2, TR_3, and TR_4 are illuminated by lamps L_1, L_2, and L_3, and in the quiescent state relays RL_A, RL_B, and RL_C, are energised and relay contacts $RL_A/1$, $RL_B/1$ and $RL_C/1$ are open. A falling drop operates the appropriate relay which, in turn, actuates the corresponding channel of the flow pen event recorder. S_2 is an event marker. The power for the instrument is provided by a basic mains-operated 24 volt stabilised power supply.

Using this equipment, saliva was collected from 3 volunteer normal subjects. An 18 cm. length of soft polyethylene tubing, internal diameter

1.02 mm. linked the collecting device (Carlson-Crittenden cup or Polyethylene Catheter as described in Chapter 1) to the top of the drip chamber. A variety of stimuli was applied and the saliva collected at different flow rates. The stimuli included lemon juice, salt, oxo, sweets and chewing paraffin wax (Figure I, 8). The salivary volume was measured by weight as described (page 8).

The effect of irregular application of the stimulus, chewing, swallowing, and mouth movements, was studied.

Iodine was estimated on samples of the saliva collected at different flow rates by the method described in chapter 9.

RESULTS

Using the equipment described and illustrated in Figures I, 9 and I, 10, the pattern of parotid and submandibular salivary flow studied under 'resting' conditions and after various stimuli in one subject is shown in Figure I, 11. The rate of flow during each period was relatively constant. A graded increase in flow rate occurred with chewing paraffin wax, holding and sucking a sweet ('boiling') and lemon juice. A record of drop rate will, however, only represent accurately the pattern of salivary flow if the drop size does not vary. It was found that at any one flow rate the drop size remains relatively constant. Table I, 1 shows the results in 3 subjects from whom parotid

saliva was collected at three different flow rates, 'resting', after a sweet ('boiling') and lemon juice stimulation. Although drop size tended to increase with increasing flow rate, such differences were negligible in comparison to the proportional changes in flow rate.

If care is not taken in the collection of saliva and application of stimuli the rate of salivary flow may be irregular and patterns such as shown in Figure I, 12 obtained. It can be seen that swallowing causes a transient increase in salivary flow. The effect of lemon juice may persist for some time after the stimulus has stopped. Sucking a 'boiling' and changing its position in the mouth may alter the flow rate. Irregularly applied stimuli result in an irregular rate of flow.

The iodide concentration in the parotid and submandibular saliva collected at different but <u>regular</u> flow rates is shown for one subject in Figure I, 13. There is a negative relation between the salivary iodide concentration and the rate of salivary flow. As the salivary flow rate increased the concentration of iodide in both parotid and submandibular saliva decreased. Figure I, 14 in contrast shows the iodide concentration of parotid and submandibular saliva at different flow rates when the pattern of salivary flow is <u>irregular.</u> When compared to Figure I, 13 the relation between flow rate and iodide concentration is less definite.

DISCUSSION

This photo-electric system confirms that there are many variables affecting flow rate, e.g. swallowing, chewing, sucking, the position of the stimulus in the mouth, the rate of application of the stimulus and the time interval following the previous stimulus. It is desirable, therefore, to monitor the pattern of salivary flow during each collecting period. This system allows the pattern of flow to be recorded and the saliva to be collected for subsequent analysis. Where the pattern of salivary flow is irregular, only the mean flow rate is obtained when the total volume of the sample is divided by the collection time. Inaccurate results will then be obtained as has been illustrated using iodide concentration as an example. Samples should therefore be discarded where the rate of flow is noted to be grossly irregular.

A photo-electric salivary drop counter has been previously described by Bates (1958). This apparatus was designed for laboratory use only to measure the salivary flow rate from one gland. Bates found it inaccurate because of the mixing of air bubbles with the saliva when using a Carlson-Crittenden cup. In the apparatus described here, because of the drip chambers, air mixed with saliva is not usually a problem. When it does occur it can be overcome by (a) adjusting the air suction pressure to improve retention of the suction cup or, (b) using the Kerr method of

combined intraduct catheterisation and suction cup as described in Chapter 1. If the pattern of drop rate is regular the drop size remains constant and accurate measurement of flow rate can be obtained by drop counting. The flow rate recorder described here is portable and the photoelectric drop counters can be positioned near the patient's mouth. Secretions can be monitored simultaneously from three salivary glands and the saliva can be readily collected for subsequent analysis in bottles placed underneath the drip chamber. A permanent record of salivary flow is made by the electronic 4-track recorder which can be situated at some distance from the patient.

CHAPTER 3

THE SELECTION OF SALIVARY STIMULI

The type of saliva which should be collected for a particular investigation varies. 'Resting' saliva can be selected because it is more representative of the secretion present throughout most of the day and night. Stimulated saliva is required when a maximal response is being examined. In order to study the metabolism of any constituent fully, saliva collected at a range of flow rates is desirable.

Wide variations in 'resting' flow rate occur between individual patients (Kerr 1961) and to collect 1 ml of 'resting' parotid saliva may take 30-45 minutes. On the other hand, after stimulation, the salivary flow rate varies with the rate of application of the stimulus, the position of the stimulus in the mouth, and chewing and swallowing as already demonstrated. The different effect on flow rate when chewing on one side and then the other described by Kerr (1961) is strikingly illustrated in Figure I, 15. Some workers have used a chemical means of stimulation, as has been used successfully in tests of gastric function (Kay 1953). Pilocarpine, a parasympathomimetic drug, has been administered orally (Dawes 1966), subcutaneously (Kullander and Sonesson 1965) and intravenously (Curry and Patey 1964). Methacholine similarly has been used (Diamant et al 1957 and Thaysen et al 1954). Baxter (1933) in dogs, has however shown that pilocarpine is not a suitable

physiological stimulus as lowered concentrations of sodium and potassium occur using this method. Dawes (1966) confirmed Baxter's observations in man and emphasised that pilocarpine stimulation does not reproduce the normal combined parasympathetic and sympathetic control. Furthermore, parasympathomimetic drugs in sufficient dosage to produce very high flow rates can produce undesirable side effects such as flushing, palpitations, colicky abdominal pains, and an urgent desire to micturate (Curry and Patey 1964) and in some disease states they would obviously be contra-indicated.

A complication associated with the collection of saliva is the possible occurrence of 'rest transients'. It was observed that the concentration of potassium in saliva secreted at the start of a period of stimulation was greater than the concentration found after secretion had continued for a minute or two (Kestyus and Martin 1937). This phenomenon has been demonstrated for potassium and iodide in the dog submaxillary gland (Burgen and Seeman 1958).

In view of the above findings in the subsequent work reported here routine saliva collection procedure was developed as follows:

1) The subject or patient was seated comfortably in a dental chair and the procedure was explained to him.

2) The collection device was applied. 5 drops of lemon juice were then applied to the tongue.

This initial stimulus flushed out stagnant secretions and the saliva collected during the next 15 minutes was then discarded to avoid 'salivary rest transients'.

3) Saliva was then collected for analysis under conditions of minimal stimulation or 'resting' and using several different intra-oral stimuli. For many studies saliva collected (a) 'resting' for 30 minutes, (b) sucking a fruit gum* for 5 minutes and (c) lemon juice** stimulation for 2 minutes was sufficient. In each experiment the order of collection of (a), (b), and (c) was varied. Stimuli were applied for at least 2 minutes before the collection of a sample was commenced.

Using this technique it was found that:-

I Saliva was obtained at low, medium and high flow rates (Figure I, 16).

II A normal range of flow rates could be defined for 'resting', fruit gum, and lemon juice stimulation (Table I, 2).

III A good correlation was obtained between the right and left parotid glands in normal subjects (Table I, 3), and

* Rowntree's Fruit Gums (Rowntree & Co. Ltd., York, England)

**'Jif' Lemon Juice (J. & J. Colman, Ltd., Norwich, England).

IV That reproducibility was satisfactory for the individual subject provided saliva was collected at the same time of day and at approximately the same time interval after the last meal (Table I, 4).

CHAPTER 4

SIALOGRAPHY

Sialography was introduced as a diagnostic procedure by Bársony in 1925. It is a useful technique in the diagnosis of calculi, duct strictures, chronic inflammatory conditions, fistulae and neoplastic disease of the salivary glands. Because of their superficial position, radiological examination is particularly suitable for the investigation of salivary gland disease. Adequate demonstration of the duct system and where possible, the acini, form the essential part of the examination. As in other radiographic methods a high degree of diagnostic accuracy can only be obtained if clinical and radiological findings are considered together.

Few modifications to the original hand injection technique described by Bársony have been described. Fine rubber catheters were used instead of metal cannulae by Putney and Shapiro (1950) and tapered polyethylene tubing by Liverud (1959). Early workers used barium suspensions and potassium iodide which were replaced by fat soluble media (e.g. Lipiodol). Because of the relatively high viscosity these media were expelled from the ducts slowly over a period of 1-3 days (Ollerenshaw and Rose 1951). The vehicle of 'Lipiodol,' poppy seed oil, has irritant properties and foreign body reactions have been described in dogs (Epsteen and Bendix 1954). Recently the new water soluble contrast media have been available but were not widely used initially because of their low viscosity and rapid emptying time

from the ducts. It was necessary, therefore, to use a 'closed system' of hand injection technique, the needle or catheter being maintained in position while the radiographs were exposed (Rubin and Holt 1957). In 1958, Gullmo and Böök-Hederstrom described a hydrostatic technique in which the contrast medium is allowed to flow into the gland using only the force of gravity. A modification of this method has been suggested by Drevattne and Stiris (1964).

Sialography was used in the present studies as one of the screening tests in patients who had complained of xerostomia and where Sjögren's syndrome was suspected (Part IV, Chapter 30). It was clear early in these investigations that the conventional method of performing sialography, i.e. by hand injection technique, was unsatisfactory for use in established cases of Sjögren's syndrome. Where destructive changes had occurred within the salivary glands the pain sensation normally felt when the ducts are distended was absent in these patients. Thus overdistension and extravasation of radiopaque medium into the surrounding tissues was a not infrequent occurrence. Figure I, 17 demonstrates the retention of contrast medium in the adjacent tissues in a patient with Sjögren's syndrome who presented with a history of sialography 6 months previously.

Ideally for sialography the radiopaque contrast medium should have similar physical characteristics to saliva. It should be introduced into

the duct at a constant pressure just greater than the secreting pressure of the salivary gland. Two investigations were therefore carried out:-

1) A comparison of the physical characteristics of the radiopaque media available with saliva. (Table I, 5).

The viscosity of three different contrast media was measured using a Brookfield Rotational Cone and Plate microviscometer.

The specific gravity values quoted were provided by Glaxo Laboratories Ltd., Greenford, Middlesex (Triosil '45') and Pharmaceutical Specialities Ltd., (Neohydriol Fluid and Viscous).

The results are shown in Table I, 5 with mean values reported for 'resting' parotid and submandibular saliva (Schneyer 1955 and Kerr 1961).

It is clear that the water soluble contrast media are most similar to saliva with regard to the physical properties assessed.

2) The measurement of the secreting pressure of the parotid and submandibular glands in a group of eighteen patients was performed using the simple apparatus shown in Figure I, 18. This consisted of a standard metre stick arranged vertically with a polyethylene tube (1.5 mm. in diameter) attached anteriorly. This was then fixed to a blood transfusion stand. The lower end of the metre stick was adjusted for each subject, to lie at the level of the gland being studied. The combined catheter and cup method described by Kerr (1961) was used for collection of parotid saliva and for the submandibular, a

tapered polyethylene catheter was inserted into the duct. The tube collecting the saliva was connected to the polyethylene tube attached to the metre stick. 'Resting' levels were obtained and then stimulated levels (sucking a fruit gum); the means and S.E.M. are shown in Table I, 6. The mean 'resting' parotid secreting pressure was 16.4 ± 1.48 and increased to 63.5 ± 1.41 cms H_2O after stimulation. The corresponding figures for the submandibular gland were very similar 17.1 ± 0.97 to 69.6 ± 1.26 cms H_2O.

The author is not aware of any previous figures reported which would allow a strict comparison to be made.

Based on these findings (1 and 2) a sialographic technique was devised whereby a water soluble contrast medium (Triosil '45') was introduced into the salivary duct system at a constant pressure of 70-90 cms of H_2O. The latter was achieved using a 20 cc syringe and tubing set at a height of 70-90 cms above the gland to be investigated. At this pressure filling of the gland occurred in about 5-10 seconds and overfilling rarely occurred.

This technique differs from that of Gullmo and Book Hederstrom (1958) who used a pressure of 20-40 cms H_2O and maintained the catheter in position by attaching it to the oral mucosa with a metal clip. Drevattne & Stiris (1964) use a similar pressure to that described here and employ a nylon introducer with the catheter.

EQUIPMENT. (Figure I, 19).

A 20 ccm. Glass Syringe Barrel.

Two Catheters - 1) Portex polythene tubing P.E. 205 100 cm. in length.

2) Portex polythene tubing P.E.160 finely tapered at one end, 25 cm. in length.

Two metal connectors.

Adaptors and tap for polythene tubing, size P.E. 205 with Luer-Lock fitting.

Adaptor and tap especially designed with a screw cap to fit tubing P.E. 205 at one end and to take tubing P.E.160 at the other end.

Lacrimal probe dilator.

Elema-Schonander skull table.

No. 13 cone with a slit diaphragm.

8 x 6 Ilford Blue brand film with fast tungstate screen.

Sodium metrizoate 45 per cent (Triosil '45').

Slices of fresh lemon.

METHOD

Identical procedures were carried out for all patients. The details of patients examined are included in the chapters reporting the results of the present studies (Chapters 30, 31, and 33).

1: HISTORY AND CLINICAL EXAMINATION

2: PLAIN FILMS:

Plain radiographs were taken first to demonstrate sialolithiasis, calcification, or gland enlargements.

3: SIALOGRAPHY:

a) Passive filling phase: With the apparatus assembled, the syringe barrel is filled with contrast material and set at a height of 70-90 cms above the level of the patient's mouth (Figure I, 20). The medium is allowed to run through the catheter system freely to expel all air bubbles. The duct orifice is located with a lacrimal probe and where necessary, gently dilated (Figure I, 21). The tapered end of the catheter is then introduced 0.5 to 1 cm. into the duct (Figure I, 22) and the patient asked to grip the catheter gently with the lips. Before commencing the examination, it is briefly explained to the patient, who is asked to indicate when discomfort or pain is felt, by raising the right hand. At this point the exposure is made at once while the contrast agent is flowing. The tap is then closed to prevent unnecessary distention of the gland. The same

procedure is repeated for each view. The short catheter may then be replaced by a fresh one and contrast material run through it. The apparatus is then ready for the next examination.

b) Active Emptying phase: The patient is given a slice of lemon to suck immediately after the passive filling phase is completed, and the catheter is removed from the mouth. After a five-minute interval further exposures are obtained. A normal secretory phase film shows no evidence of residual contrast medium.

Examples of the results obtained in normal and pathological glands are shown in Figures I, 23 - I, 33.

This hydrostatic method was found to be superior to the conventional hand injection techniques for the following reasons:

1) Constant pressure is obtained during the introduction of the radiopaque medium and overfilling is a rare occurrence as compared with the hand injection technique.
2) As films are exposed during the filling phase no reflux of contrast medium occurs.
3) A water soluble contrast medium can be used which is rapidly expelled from the duct system. One gland examination including the emptying phase takes 15-20 minutes and several glands may be examined at the one visit. In comparison, contrast media containing iodised oils are retained for 1-3 days (Schultz and Weisberger 1947, Ollerenshaw and Rose 1951).

CHAPTER 5

THE USE OF RADIOISOTOPES TO STUDY SALIVARY GLAND FUNCTION

Isotopes have been used extensively to study salivary gland function in animals (Burgen and Seeman 1957, and 1958; Cohen et al 1955; Cohen and Myant 1959; Towbin and Perkins 1958).

Radioisotopic methods have also been used to study human salivary gland function in health and disease. Following the administration of the isotope it is secreted in the saliva and by collecting samples of blood and saliva it is possible to measure the saliva/plasma ratio. Several workers have measured the saliva/plasma ratio using ^{131}I (Schiff et al 1947; Honour et al 1952; Freinkel and Ingbar 1953; Ferguson et al 1956; Cohen and Myant 1959). The effect of drugs on this ratio has also been studied (Rowlands et al 1953; Edwards et al 1954; Ferguson et al 1957; Myant 1960). Gabrielson and Kretchmar (1956), Fellinger et al (1956) have studied the saliva iodide trap in different thyroid states using ^{131}I. Uptake of radioiodine by the salivary glands has been studied (Negri and Pochin 1961). It is also possible to obtain further information about the chemical nature of the salivary iodine using radioisotopic methods (Fellinger et al 1956; Cohen and Myant 1959; Cohen 1962; Alexander et al 1966; Papadopoulos et al 1966.) Awwad (1959) has used ^{131}I to study the salivary gland iodide

concentrating mechanism in a group of patients following external radiation. Estimations of the plasma inorganic iodine a quantity too small to be measured chemically have been made from the specific activity of the salivary iodide following a tracer dose of radioiodine (Gerbaulet and Maurer 1958, Fitting 1960 and Harden et al 1965a).

Using radioactive phosphorus, Stevens (1953), measured the secretion of this element and its rate of appearance in saliva.

Some aspects which have been studied in more detail in the present work are:

1) The Saliva/plasma ratio of iodine. This may be used as a clinical test to screen patients suspected of having a type of dyshormonogenesis in which failure of iodine trapping is shared by salivary, thyroid and gastric glands (Chapter 28). Normal ranges for the saliva/plasma ratio at different salivary flow rates are detailed in Chapter 20.

2) The use of the specific activity principle to derive indirectly the plasma inorganic iodide concentration (PII) a quantity too small to be measured directly (see below).

3) Combined Stable and Radioiodine studies. Whenever possible stable iodine (^{127}I) and radioiodine studies (^{132}I or ^{131}I) were combined. As Riggs (1952) has pointed out isotopes are ideal for the measurement of the proportion of body iodine which follows a particular metabolic pathway but

chemical methods give information about the absolute quantities of iodine involved. Only by combining the two techniques can a complete understanding of iodine metabolism be obtained.

4) Combination of isotopes Tests using combinations of isotopes were occasionally used to compare the salivary concentrating mechanism for each isotope. The isotopes were administered simultaneously and as the radioactivity is counted for each isotope in the same saliva sample, the variable of flow rate is excluded.

Isotopes Used

The isotopes used were ^{131}I, ^{132}I, ^{82}Br and $^{99m}TcO_4$. Their physical characteristics and radiation dosages are described in Table I, 7. The advantages of using ^{131}I and ^{132}I are that they follow exactly the metabolic pathway of stable iodine (^{127}I) and introduce no significant amount of iodine into the organism. They therefore act as true tracers and can be used to study the salivary iodide concentrating mechanism in a similar way to the thyroid. ^{131}I has a half life of 7-9 days and ^{132}I a half life of 2-3 hours. Used as clinical tracers ^{131}I is given in a dose of 5 μc and ^{132}I in a dose of 50 μc.

Pertechnetate ^{99m}Tc has some similar biological properties to isotopes of iodine. It is also concentrated by the salivary glands. Because of its short half life of 6 hours and its low primary particle radiation it is

being increasingly used as a clinical tracer in human studies (Chapter 5). It is of particular interest because it is trapped by the thyroid gland but not subsequently bound to protein and therefore it may be used in comparing the salivary and thyroid iodide trapping mechanism. ^{82}Br was also studied because, like iodide and pertechnetate, bromide is an anion of the VIIth periodic group. It has been reported by Wolff (1964) using tissue slice experiments in mice, that the salivary glands concentrate other anions of this group, as well as iodide.

RADIOISOTOPE PROCEDURES USED IN THE SALIVARY STUDIES (reported in Part III).

In most of the studies reported ^{132}I was used. A tracer dose of 50 μc was given orally to subjects while fasting. Urine was collected from 60 to 150 minutes from the commencement of the test. Saliva was collected for periods varying from 2-30 minutes depending upon the stimulus. The blood sample (10 mls) was taken at the midpoint of the urine and saliva collections, i.e. 105 minutes. The radioactivity of this plasma sample was assumed to be the mean radioactivity of the plasma from 60 to 150 minutes (Alexander et al 1962). Furthermore the radioactivity of the plasma sample taken at the mid-point of the saliva collection was assumed to correspond with the mean radioactivity of the saliva since, as shown below, no significant lag period was found between the presence of the istotope in the plasma and its appearance in the saliva. To confirm this saliva samples

were collected at 10-second intervals following the intravenous injection of ^{132}I and $^{99m}TcO_4$. Maximum radioactivity was found 2½ minutes after the intravenous injection of the isotope. Since the flow rate was 0.5 ml/min and the dead-space estimated as being approximately 1.3 ml., it can be assumed that the isotope appears in saliva almost instantaneously.

Using the above method the following parameters were derived -

a) Salivary iodide clearance.

b) Absolute amount of iodide secreted in saliva.

c) Saliva/plasma iodide ratio.

d) Plasma inorganic iodine.

a) The Salivary iodide clearance is the volume of plasma completely cleared of its iodide content by a salivary gland per unit of time. In the present work the salivary clearance is expressed in ml/min and is calculated in the following way:-

$$\text{Salivary iodide Clearance (ml/min)} = \frac{\text{Saliva } ^{132}\text{I/per cent dose/ml} \times \text{Vol. of Saliva (ml)}}{\text{Plasma } ^{132}\text{I/per cent dose/ml} \times \text{Duration of Collection (min.)}}$$

b) The Absolute amount of iodide secreted in saliva is the absolute quantity of iodide secreted by a salivary gland in unit time. In these studies this quantity is expressed in µg/hr and is calculated -

$$\text{Absolute amount of iodide secreted in saliva (µg/hr).} = \frac{\text{Saliva I (µg/100 ml)} \times \text{Volume in 1 hr (ml)}}{100}$$

c) The Saliva/plasma iodide ratio is the ratio of the iodide in saliva in μg/100 ml to the plasma inorganic iodide in μg/100 ml. Usually it is obtained after a tracer dose from the ratio of radioiodine in saliva (% dose/ml) to the radioiodine of the plasma (% dose/ml).

$$\frac{\text{Saliva } ^{132}\text{I}}{\text{Plasma } ^{132}\text{I}} = \frac{\text{Saliva } ^{132}\text{I (\% dose/ml)}}{\text{Plasma } ^{132}\text{I (\% dose/ml)}}.$$

d) The plasma inorganic iodine is the concentration of iodide present in the plasma and is measured in μg/100 ml. It is too small a quantity to be measured directly but can be derived indirectly using the specific activity method first applied to the urine by Stanley (1949) and later to saliva (Gerbaulet and Maurer 1958). The methods are based upon the principle that after a tracer dose of radioiodine the specific activities of the plasma, saliva and urine are all equal.

Thus:

$$\frac{^{132}\text{I Saliva}}{\text{Salivary iodide}} = \frac{^{132}\text{I Plasma}}{\text{Plasma inorganic iodine}} = \frac{^{132}\text{I Urine}}{\text{urinary iodide}}$$

The iodide in both saliva and urine can be measured chemically and therefore the PII can be derived -

$$\text{PII (μg/100 ml) (from specific activity of urine)} = \frac{\text{Urine I (μg/100 ml) x Plasma } ^{132}\text{I(per cent dose/ml)}}{\text{Urine } ^{132}\text{I (per cent dose/ml)}}.$$

or

$$\text{PII } (\mu g/100 \text{ ml}) \text{ (from specific activity of saliva)} = \frac{\text{Saliva I } (\mu g/100 \text{ ml}) \times \text{Plasma } {}^{132}\text{I} \text{ (per cent dose/ml)}}{\text{Saliva } {}^{132}\text{I} \text{ (per cent dose/ml).}}$$

2) Estimation of Saliva/plasma ratios of isotopes of the VIIth periodic group used in combination.

A solution containing a mixture of radioisotopes ^{82}Br, ^{132}I and $^{99m}TcO_4$ was injected intravenously into the volunteer subject. Saliva (parotid and/or submandibular) was collected at three flow rates using the methods already described. At the midpoint of each salivary collection a plasma sample is obtained by venepuncture. Plasma and salivary samples are counted using a nuclear Chicago automatic well-type counter. Each plasma and saliva sample is counted on three occasions. Firstly, ^{132}I plus ^{82}Br were estimated, excluding $^{99m}TcO_4$. Secondly, this count was repeated after decay of ^{132}I giving an estimate of ^{82}Br. Thirdly, the counting conditions were adjusted and ^{82}Br and $^{99m}TcO_4$ estimated. Counts for each individual isotope were obtained by solving three simultaneous equations using a Sirius computer. Salivary/plasma ratios were then calculated for each isotope at the three flow rates.

CHAPTER 6

SALIVARY GLAND UPTAKE AND SCANNING USING PERTECHNETATE - ^{99m}Tc

Radioisotopic visualisation or scanning is a diagnostic technique which has been increasingly used during the past decade. The basis of this method is that according to their physiological and metabolic functions, organs and tissues may contain different concentrations of a suitable isotope after it has been administered as a clinical tracer (Figure I, 34). This difference in radioactivity between an organ and surrounding tissues can be recorded and charted using a Picker Magnascanner V (Figures I, 35 and I, 36).

Using this principle various isotopes have been used to visualise many different organs including brain, liver, kidney, spleen, pancreas, stomach, bone, lymph nodes, lungs and placenta (Mallard 1966). Scanning can thus be used as an additional diagnostic aid in the detection of disease or of a tumour mass within an organ. It may also be of value in the location of tumour metastases. Because of its iodide concentrating ability the thyroid gland is particularly suitable and scanning is now a standard technique in the diagnosis of thyroid disease. Although the salivary iodide concentration mechanism is well recognised little information is available concerning the actual uptake of iodine by the salivary glands (Negri and Pochin 1961). Counting procedures are difficult because of problems in

isolating one salivary gland and a further problem is, of course, the radiation dosage to the patient. A detailed study of the trapping mechanism is however possible using a scanning technique with $^{99m}TcO_4$ which, as shown in Chapter 20, like radioiodine, is concentrated by the salivary glands. Technetium ^{99m} is an almost ideal scanning agent: its short half-life (6 hours) coupled with the near absence of particulate radiation permits relatively large doses to be administered with little hazard to the patient and the 140 KeV gamma-radiation is about optimum for scanning. Well defined scans can thus be completed quickly.

In this chapter studies of uptake and scanning using $^{99m}TcO_4$ by the submandibular and parotid glands in man will be described.

METHODS

Eight subjects were studied aged 31 to 60 years. All were volunteers and none had evidence of salivary gland disease. A tracer dose of 800 μc of $^{99m}TcO_4$ was injected intravenously. The resulting radiation dose to the salivary and thyroid glands was less than 0.2 rad. Using the Picker Magnascanner V the subjects were scanned in the antero-posterior position over an area between the bridge of the nose and the sternal notch. This region which includes both the salivary glands and thyroid gland was scanned 30-35 minutes after the tracer dose. A line spacing of 0.9 cm and a scan speed of 100 cm/min. was used. Each scan took approximately 5 minutes.

A phantom containing a known dose of the injected isotope was scanned under identical conditions. Scans more suitable for photographic purposes were obtained by adjusting the speed to 40 cm/min and the line spacing to 0.5 cm. Such scans took approximately 20 minutes. Circles were drawn on the scan around the thyroid and salivary glands and the dots enclosed were counted. Circles of the same diameter were drawn in an adjacent area and a correction for radioactivity in underlying tissue and also for room background was made by subtracting the latter dot-counts from the counts over the gland as described by Andros et al (1965). By comparison with the scan of the standard it was possible to express the net dot-counts over the glands as percentage of the injected dose. Inaccuracies may arise from a statistical counting error and from estimation of the background activity. The error is approximately $\pm$ 0.05% dose. (I am grateful to Mr. T. Hilditch, Western Regional Physics Department, who developed this method for the counting procedure.)

Two patients were scanned 3 minutes after administration of the tracer dose and the scans were repeated at intervals up to 105 minutes. Potassium perchlorate (500 mg) was administered orally at 65 minutes. In one patient the mouth was rinsed with water between 95 and 100 minutes in an attempt to remove the radioactivity present in this region.

One patient was scanned as described above and, on a second occasion, atropine 0.6 mg was given subcutaneously 30 minutes before administration of the tracer dose.

RESULTS

The uptake of $^{99m}TcO_4$ by the salivary and thyroid glands at approximately 35 minutes is shown in Table I, 8. The thyroid gland takes up consistently more $^{99m}TcO_4$ than each individual salivary gland ($P < 0.01$). The thyroid gland took up between 0.34 and 1.51% dose, mean 0.96 $\pm$ S.E.M. 0.174. This was consistently higher in each patient than the uptake in the individual salivary glands which ranged from 0.04 to 0.80% dose. In patients in whom both submandibular and parotid glands were scanned the uptake by the submandibular gland was less than the uptake by the parotid gland. At 35 minutes the activity present in the mouth region ranged from 0.06 to 0.66% dose. A typical scan taken at a speed of 40 cm/min. is shown in Figure I, 37. Radioactivity can be clearly seen in the thyroid, both submandibular and both parotid glands and centrally in the 'mouth' region.

Serial uptake measurements by the submandibular glands are shown in Figure I, 38. The uptake over the two submandibular glands was almost identical and the mean values are shown in the figure. This reached a peak of 0.7% by 47 minutes. The activity in the 'mouth' region was negligible over the first 10 minutes but by 24 minutes it accounted for 0.2% of the dose,

thereafter rising rapidly reaching 1.2% of the dose by 57 minutes. The thyroid uptake reached a maximum of 1% of the dose by 13 minutes. Twenty-five minutes after the administration of 500 mg potassium perchlorate orally, the radioactivity over the submandibular gland regions could not be distinguished from background. In contrast over this period the activity in the 'mouth' region rose from 1.2% to 1.8%. Attempts to remove this activity in the mouth by rinsing with water failed.

Serial uptake measurements in a second patient are shown in Figure I, 39. At each time interval the mean uptake over the parotid glands was slightly higher than the mean uptake over the submandibular glands. In this subject the radioactivity in the 'mouth' region prior to perchlorate administration never exceeded 0.23% of the dose. The thyroid gland uptake reached a maximum of 1.5% at 25 minutes. After the administration of potassium perchlorate the parotid and submandibular gland uptakes fell from 0.44% and 0.30% respectively to negligible amounts.

In Figure I, 40 are shown the salivary and thyroid glands before and after perchlorate administration. Before perchlorate the submandibular and parotid glands are clearly outlined. After perchlorate, radioactivity is seen only in the 'mouth' region.

In one patient a tracer dose of $^{99m}TcO_4$ was given intravenously 30 minutes after subcutaneous injection of atropine 0.6 mg. The thyroid

and salivary glands were scanned 12, 23 and 132 minutes later. The scan obtained between 23 and 43 minutes is shown in Figure I, 41a. When compared to Figure I, 37 it is apparent that there is no significant difference in the uptake of the isotope over the thyroid and salivary glands. Considerably less activity is present however in the 'mouth' region in the scan obtained after atropine. At 18 minutes the mean submandibular gland uptake without atropine was 0.45% of the dose, with atropine 0.44% of the dose. The values for the parotid gland were 0.59% without atropine and 0.53% with atropine. At 40 minutes the values were also similar, submandibular 0.53% without atropine and 0.52% with atropine, parotid 0.77% without atropine and 0.77% after atropine. By 147 minutes the activity in the salivary glands after atropine had fallen considerably but the activity in the 'mouth' region had increased to 1.84% of the dose (Figure I, 41b).

DISCUSSION

In recent years the isotope $^{99m}TcO_4$ has been used to scan various organs in the body including thyroid, brain, liver, stomach, heart and spleen (Mallard 1966). Little attention has been paid however to the salivary glands. In this chapter the salivary and thyroid glands have been scanned with technetium. This will permit detection of heterogeneous function in the salivary glands in the same way as has been possible in the thyroid gland. Furthermore, it has been possible to quantitate the uptake of the isotope by

these glands. No previous results of uptake and scanning of the salivary glands have been reported in the literature. However, the thyroid uptakes reported are in agreement with values previously published by Andros et al (1965). From the present study it appears that the uptakes over individual salivary glands are significantly less than the thyroid uptake but that the combined salivary gland uptake may exceed the uptake by the thyroid gland. Harden et al (1965b) have previously shown that the absolute amount of iodine excreted in saliva from the two parotid and two submandibular glands was of the same order as that taken up by the thyroid. Moreover, the same authors found that more iodine was secreted in the parotid saliva than in the submandibular saliva which is in agreement with the $^{99m}TcO_4$ uptake measurements in the present study.

Although the uptake of $^{99m}TcO_4$ by the salivary glands has been referred to, the actual site of the isotope within the salivary glands is uncertain. There is considerable evidence that iodine is concentrated in the cells of the salivary ducts (Burgen and Seeman 1957; Burgen et al 1959; Cohen et al 1955). In addition to any activity present in the duct cells, $^{99m}TcO_4$ secreted into the duct lumen in saliva will also be seen in scans.

Activity can be seen on the scan situated centrally in the 'mouth' region. If this were all in saliva, the equivalent volume of saliva could exceed 10 ml. Since the volume of saliva in the mouth is considerably less and since the activity is not removed by rinsing the mouth with water, one

must postulate that either the $^{99m}TcO_4^-$ is adsorbed on to the oral mucosa or that it is concentrated by the minor salivary glands. That the adsorption on to the mucosa is the more important factor is suggested by the facts, a) the activity increases more slowly with time after the tracer dose than the uptake over the parotid or submandibular glands, b) its appearance is much delayed when saliva secretion is decreased by prior atropine administration, c) it is not discharged by perchlorate. Furthermore, we have shown that a similar appearance can be obtained on scanning after a tracer dose of $^{99m}TcO_4^-$ is contained in a mouth rinse.

It is well known that after perchlorate administration the concentration of iodine in saliva falls (Rowlands et al 1953; Ferguson et al 1957; Myant 1960; and Mason et al 1966b). This study shows as one might expect on theoretical grounds, TcO_4^- is completely discharged from the salivary glands by potassium perchlorate. Negri and Pochin (1961) have previously claimed, using a profile counting procedure, that following administration of perchlorate, although a fall in salivary radioiodine concentration occurred, the radioactivity was not discharged from the salivary glands. They failed to find a satisfactory explanation for this observation. In the light of the present work it seems likely that the isotope present after the perchlorate administration was not in the salivary glands but in the 'mouth' region. This activity is unaffected by perchlorate administration at a time when the uptake over the salivary glands has fallen to background

levels (Figures I, 38 and 39).

The above studies have some clinical implications. Whitley et al (1966) have reported visualisation of extracranial neoplasms using $^{99m}TcO_4$. They found however that the thyroid, salivary glands and stomach might interfere with the visualisation of tumours in their vicinity. Potassium perchlorate by suppressing uptake of $^{99m}TcO_4$ by these glands might be of value in tumour scanning if given prior to the administration of the isotope. Scanning of the neck of patients with thyroid carcinoma is of value in detecting the presence of residual functioning tumour tissue or lymph node metastases. Unless one is aware that the salivary glands also concentrate technetium and iodine one may mistake salivary gland uptake for tumour metastases. Skanse et al (1961) have for example reported a patient in whom the right submandibular gland was removed at operation having been mistaken for a functioning thyroid tumour metastases.

There are many situations where an assessment of salivary gland size, position, and function would be of value. Scanning has the advantage of speed, little inconvenience to the patient and minimal radiation dosage. Wider experience must be gained, however, before the precise role of this technique in the investigation of salivary gland function and disease can be established.

SUMMARY

Pertechnetate - ^{99m}Tc is concentrated by the salivary and thyroid glands in man. The uptake by the submandibular, parotid and thyroid glands can be quantitated using a radioisotope scanning procedure. At 30 - 35 minutes the thyroid gland uptake was significantly higher than that of any one salivary gland but the uptake by the four salivary glands frequently exceeded the thyroid uptake.

Potassium perchlorate completely discharged the isotope from the salivary glands.

Some radioactivity was present centrally in the 'mouth' region and it is concluded that this was due, at least in part, to adsorption of the isotope onto the oral mucosa.

Scans using $^{99m}TcO_4$ or ^{131}I to locate tumours in this region must be interpreted in the light of these findings.

CHAPTER 7

AUTORADIOGRAPHY

Autoradiography is a method by which the presence of radioactive material may be detected in a specimen of tissue or cell sample. The aims of this method are:-

1) to detect the presence of the isotope.
2) to locate its presence within a tissue or cell.
3) to analyse with the aid of histochemical technique the form in which the radioactive label is present.

The method consists essentially of bringing the sample containing the radioactivity into juxtaposition with the photographic emulsion. The photographic emulsion contains silver halide crystals imbedded in gelatin. These silver halide crystals have some 'imperfections' built into them called 'sensitivity specks' or 'electron traps'. Energy which is released from a tissue containing a radioisotope is capable of breaking lattice bonds within the crystals and will set free negatively charged electrons. These electrons are trapped by the electron traps creating a field of electronegativity around them. With the breaking of the lattice bonds positively charged silver ions are set in motion also and these collect around the negatively charged electron traps and are reduced to metallic silver. Any photographic crystal containing such silver specks is a crystal with a latent image which can be

developed to visibility with ease. The metallic silver filaments form an irregular grain larger than the original crystal and these are the photographic 'grains' seen in a developed photographic negative under suitable magnification (Lajtha 1961).

Conventional autoradiographic techniques have been applied to many problems since the pioneer work in the 1940's of Leblond (1943) Gorbman and Evans (1941) Doniach and Pelc (1949). Much work has been carried out on the localisation and speed of metabolic pathways in the thyroid gland using isotopes of iodine (Wollman and Wodinsky 1955). Here the iodide trapped is subsequently bound to protein and thus remains in its site of activity when the tissue is removed. In contrast, however, the iodide which is also concentrated by the salivary glands remains in the inorganic form. It therefore moves quickly away from its in vivo position on removal of the salivary tissue unless immediately frozen and maintained in this state until exposure to the photographic emulsion is complete. Cohen et al (1955) have described autoradiographic studies in the hamster submaxillary salivary gland using ^{131}I and freeze drying the tissue. This method was used successfully to localise the site of salivary iodide concentration to the distal ducts of the hamster. In the present study a new method described by Kintner (1965) for the localisation of diffusible labelled substances has been used. The experiments of Cohen et al (1955) have been repeated in hamsters.

METHOD

The method is based on the principle of keeping the tissue and tissue fluid contained within it solidly frozen from the time of removal from the intact animal until the exposure to the autoradiographic emulsion is completed. If this is not ensured small water soluble molecules rapidly redistribute by diffusion in all available water. The tissue on removal is therefore, immediately snap frozen using CO_2 (carbon dioxide) snow and methyl alcohol. All apparatus and equipment used including emulsion plates, containers, lightproof paper, are kept in the cryostat at -20°C for 24 hours before use. Frozen sections 10μ thick are cut in the cryostat. The usual procedure of flattening the frozen section and making it adhere to the slide by using heat from the finger tip cannot be applied here as the sections would melt with subsequent diffusion of the iodide ions. In order to avoid this complication the surface tension technique can be used.

Surface Tension Technique. This method represents a different approach to tissue emulsion contact. The principle is that the surface tension of an evaporating organic solvent (ether) containing a trace of adhesive (acrylic resin) maintains the section in position on the emulsion coated slide. Both section retention and subsequent water permeability were satisfactory when the acrylic resin was sufficiently diluted by ether to give an 0.005% solution.

The first stage was to cut about 6 frozen sections in the cryostat at approximately -17°C. The cut sections were stored temporarily on slides coated with Saran F - 120 resin (Dow Chemical Co.). The resin prevented the frozen sections adhering to the surface of the slide. All light was excluded except for the red safe light (Wratten series 1). The frozen sections were then transferred individually using a sharp metal probe to the emulsion coated scientific plates (Kodak V1056). Each section was flooded with a single drop of acrylic resin and ether (.005% solution). The drops were delivered from a capillary pipette. Once on the emulsion at -17°C the ether evaporated in less than 1 minute leaving the sections bound down by the acrylic resin. The vapourised ether was absorbed using a Petri dish of activated charcoal which was kept in the cryostat. Then the emulsion coated scientific plate with the attached sections was put in a light-tight box and stored at below -40°C for the duration of the exposure period. At the end of this time the preparation was removed from the box and placed directly in a gentle stream of 20°C air for 30 minutes in order to thaw and dry the tissue sections. After this treatment the acrylic resin held the tissue in place throughout the subsequent processing of the emulsion. After processing the removal of chemicals was affected by prolonged rinsing in running water, 10 minutes after the developer and 30 minutes after fixing. The tissue was then fixed with formaldehyde by immersing the whole preparation in a 10% aqueous

solution for 10 minutes. The formaldehyde did not visibly alter autoradiographic images in the emulsion. After a further 20 minutes water rinse, the preparation was dehydrated by passage through graded alcohols, cleared in xylol, and mounted. For the unstained tissue phase, contrast microscopy, and for the autoradiographic images, conventional light microscopy with either light or dark field illumination can be used, The results of the experiments using this technique are presented and discussed in Chapter 25.

possible complication of salivary fistula. In addition, because of their small size, which may be further decreased in some disease states, and their relationship to important neuro-vascular anatomical structures, needle biopsy is unsatisfactory.

Recently, however, in studies on children with fibrocystic disease, Warwick et al (1964) have reported involvement of the labial mucous salivary glands. Furthermore, Calman and Reifman (1966) have reported involvement of the minor buccal glands in one patient with Sjögren's syndrome and Cifarelli et al (1966) have described a patient with this condition in which the minor palatal salivary glands showed the characteristic Sjögren's histopathology.

In a group of patients with Sjögren's syndrome, labial gland biopsies were examined to evaluate their usefulness in the clinical assessment of this condition. They were compared with two control series, one of patients with rheumatoid arthritis and another of patients with non-rheumatoid polyarthritis.

The area biopsied is shown diagramatically in Figure I, 42. This site within the lower lip is richly supplied with labial mucous glands which are in close proximity to the labial mucosa (Figure I, 43). Their normal appearance is shown in Figure I, 44. The results of these studies are reported in Chapter 32.

CHAPTER 8

BIOPSY AND HISTOPATHOLOGY

The histopathology of the common pathological conditions affecting the salivary glands and ducts - obstructive, inflammatory, neoplastic, are well described in standard pathology textbooks. Complete or partial, surgical removal of diseased salivary glands is often performed. In contrast biopsy of the major salivary glands is not a technique commonly employed. This is because firstly, abnormal salivary gland conditions often have a typical history and the diagnosis may be confirmed by investigative procedures such as radiographic or sialographic methods. Secondly, if a neoplasm is suspected it is necessary to surgically explore in any case and an immediate frozen section technique may be employed at operation.

There are, however, some less common conditions affecting the major salivary glands which have recently been of considerable interest to many workers because of a possible auto-immune aetiology - Sjögren's syndrome and Mikulicz's disease.

Where these conditions are suspected it would be of considerable value to obtain a salivary gland biopsy. However, in the majority of cases this cannot be justified because of the inconvenience to the patient and the

CHAPTER 9

CHEMICAL ESTIMATIONS OF SALIVARY CONSTITUENTS

The following salivary constituents were measured chemically in various experiments and tests described in the present work. -

1) Iodine
2) Sodium and Potassium
3) Chloride
4) Uric Acid
5) Carbonic Anhydrase

1) IODINE

The method employed has been described and validated carefully by Richmond (1962) and Farrell and Richmond (1961). It may be summarised as follows:-

Chloric acid digestion destroys organic material and oxidises iodine to iodate. Iodate in the digested residue is reduced by arsenious acid and determined as iodide by its catalytic effect on the reduction of ceric sulphate by arsenious acid.

Technique: To 0.2 - 0.5 ml of saliva sample in a centrifuge tube add 5 ml chloric acid with chromate. The tube is then placed in the digestion apparatus and heated for 1½-2 hours at 160°C. Standards and a 0.5 ml water blank are

treated in the same way as the samples. At the completion of digestion approximately 0.5 ml of an amber-coloured solution remains in the tubes. On cooling, the liquid becomes colourless and deposits red crystals of chromium trioxide. Care should be taken to avoid over-digestion and the consequent loss of iodine; this is indicated by the appearance of a green colour, due to Cr+++ ion, in the residue.

Add 15 ml arsenious acid to the cooled tubes and mix thoroughly with the digestion residue by shaking. The tubes are then placed in a water bath at 37^{o}C and the contents allowed to stabilize for 10 to 15 minutes. 1 ml ceric sulphate is added to the tubes at 30 second intervals. Each tube is carefully shaken to mix the contents. After a set time (20-40 minutes) 1 ml brucine sulphate is added to terminate the reaction. The tubes are removed from the water bath and allowed to attain room temperature.

Colorimeter readings are normally taken 10 minutes after brucine addition using a 420 mμ filter. A curve of iodine content is plotted against extinction both for the standard and blank. A fresh curve is constructed for each batch of samples analysed. The results of unknowns are read off from the calibration curve.

All iodine estimations were performed in the Iodine Laboratory, Gardiner Institute, Western Infirmary.

2. SODIUM AND POTASSIUM

Sodium and potassium were measured using a flame photometer (Evans Electroselenium).

REAGENTS

Stock Standard Solutions

Stock standard solutions of sodium 120-150 m.eq/litre.

Stock standard solutions of potassium 3-6 m.eq/litre.

A Stock Solution 58.5 g NaCl/1,000 ml ≡ 1,000 m.eq/L.

B Stock Solution 7.46 g KCl/1,000 ml ≡ 100 m.eq/L.

Mixed Stock Standards (Sodium)

m.eq Na/Litre	120	130	140	150	
ml. stock soln. A	60	65	70	75	Diluted to 500 ml with ion free water
ml. stock soln. B	25	25	25	25	

Working Standards

Further dilutions each 1 : 500

Mixed Stock Standards (Potassium)

m.eq K/Litre	3	4	5	6	
ml. stock soln. B	15	20	25	30	Diluted to 500 ml with ion free water
ml. stock soln. A	70	70	70	70	

Working Standards

Further dilutions each 1 : 50

Sodium. The most satisfactory dilution was determined experimentally. Normally the saliva samples were diluted 1 in 50 for saliva collected during a resting period and 1 in 200 for stimulated saliva. In some cases however a dilution of 1 in 500 was necessary to obtain a reading on the scale.

Potassium. The most satisfactory dilution was again determined experimenta but was found to be 1 in 200 for most saliva samples.

METHOD

When carrying out series of determinations the appropriate standard solution was inserted at frequent intervals to ensure the constancy of the reading was being maintained.

The EEL flame photometer is a one cell instrument in which the galvanometer reading is proportional to the concentration of substance present..

The solutions of standards and unknowns were sprayed for 30 seconds before the readings were accepted. Up to 30 seconds there may be a slight variable decrease in the galvanometer reading. The readings remain stable after this time up to 2 minutes. This was checked as a means of testing the photocell stability.

These standard solutions are the same as those normally used for serum and this was found to be an advantage as, by varying the dilutions, serum samples could be estimated in the same batch as the saliva samples.

METHOD OF SPRAYING

Ion free water was used throughout for glass washing, preparation of standards, test samples and zero setting of the instrument. Routine laboratory gas supply at a pressure of $2\frac{1}{2}$ - 3" was employed together with compressed air at 10 lbs/sq.in Precautions were taken to allow adequate time for warming up the instrument and to avoid blocking the atomiser jet. Appropriate standard filters were used for sodium and potassium. The 150 m.eq/litre sodium standard was sprayed and the instrument adjusted until there was almost a full scale deflection - the galvanometer reading 80 divisions. Each standard was then sprayed in turn and the galvanometer reading noted. A graph was prepared of m.eq/litre against galvanometer reading. The diluted sample was sprayed and immediately after each sample the two nearest standards to give readings just above and below the sample. The tests were read off from the graph, as in Figure I, 45. Potassium was estimated in the same way using the potassium filter. The 6 m.eq/litre potassium standard was set to 60 on the galvanometer scale and the samples sprayed as described before.

CALCULATION

$$\text{Reading obtained from graph} \times \frac{\text{dilution of unknown}}{\text{dilution of standard}} = \text{m.eq/litre.}$$

3. CHLORIDE

Chloride was estimated in saliva using the mercuric nitrate method (Schales and Schales 1941).

When mercuric nitrate is added to a chloride solution unionized mercuric chloride is formed. At the end-point, the first excess of mercuric ions gives a bright purple colour with the indicator diphenylcarbazone. The titration should be carried out directly on diluted saliva rather than a protein free filtrate.

REAGENTS

Mercuric Nitrate Solution:- 20 ml. of 2N nitric acid to 2.9-3 g of mercuric nitrate with some water in a 1 litre flask. This was dissolved and then diluted to one litre with water. This solution is stable and need not be protected from light.

Indicator:- 100 mg diphenycarbazone (Eastman No. 4459) was dissolved in 100 ml of 95% ethanol. This solution was stored in the cold in a brown bottle. The solution turns yellow on exposure to daylight and even in the cold and dark it loses its colour after 2 months. The solution was prepared fresh monthly.

Chloride Standard. 584.5 mg NaCl dried at 120°C made up to one litre contains 10 m.eq/litre of Cl^-. This was used to standardise the mercuric nitrate.

Standardisation of Mercuric Nitrate

2 ml portions of standard NaCl solution plus one drop of $N_{/20}$ HNO_3^- were titrated with mercuric nitrate from a microburette calibrated to 0.01 ml after adding 0.06 ml of the diphenycarbazone. If a drop of $N_{/20}$ HNO_3^- was not added a purple colour was obtained immediately on adding mercuric nitrate thus giving a false end-point. The solution at first was brownish but on the addition of mercuric nitrate it became clear and on addition of excess mercuric nitrate it changed to an intense violet-blue colour.

Blank

Similarly blank was performed with 2 ml distilled water, one drop of 10% HNO_3 and 0.06 ml of indicator.

Test

0.2 ml saliva sample, 1.8 ml distilled water plus one drop of 10% HNO_3 and 0.06 ml of the diphenycarbazone indicator were placed in a 5 x 5/8" test tube. The titration was carried out as described for the standard until the sharp change to violet denoted the end-point. The tests were carried out in duplicate.

$$\frac{\text{Titration figure of test - titration figure of Blank}}{\text{Titration figure of standard - Blank titration figure}} \times 100 = \text{m.eq/litre chloride.}$$

4. URIC ACID

The uric acid concentration was estimated in the saliva and serum by the standard Technicon Autoanalyser method. Using this method higher values are obtained compared to the uricase method and in 13 samples it was found that $y = 0.33 + 1.17 x$ where x = value obtained using uricase method and y = value obtained with autoanalyser method.

5. CARBONIC ANHYDRASE

The Gloster (1955) modifications of the method of Krebs and Roughton (1948) was used. The estimation was conducted at a temperature of 25°C. 0.5 ml of 0.02 sodium bicarbonate solution was placed in the side arm of a Warburg Flask and 0.2 ml of the saliva sample in 2.3 ml. 0.1 M Phosphate buffer (pH 6.98) was placed in the flask itself. In a control flask 0.2 ml distilled water was used in place of the saliva. After 10 minutes equilibration the solutions were mixed and the amount of CO_2 produced was measured at minute intervals on the manometer. Each test was carried out in duplicate, the mean value at the fifth minute being taken as the enzyme activity. This was expressed in K/ml saliva (Mitchell et al 1945; Altschule and Lewis 1949).

PART II

STUDIES ON SOME CHEMICAL CONSTITUENTS OF SALIVA

PART II

INTRODUCTION

This part is concerned with studies of the chemical composition of saliva. The physiology of saliva secretion and its composition in man is reviewed briefly in Chapter 10. Some of the problems encountered in the studies and interpretation of salivary constituents are discussed in Chapter 11.

Using ordinary chemical methods it is possible to measure only a few constituents at the one time as to collect 1 ml. of parotid saliva under 'resting' conditions may take 30-40 minutes. Apart from the salivary iodide concentration which is described in detail in Part III, the following constituents of saliva have been studied.

1) Sodium, potassium and chloride - Chapter 12

2) Uric Acid - Chapter 13

3) Carbonic Anhydrase - Chapter 14

The effect of varying parotid saliva flow rate on sodium, potassium and chloride concentrations has been demonstrated by several previous workers. The present study was undertaken to evaluate the methods used (Chapter 1, 2, and 3) as well as studying the effect of flow rate on the

salivary concentrations. Uric acid concentration in parotid and submandibular saliva was studied to provide a base-line for further studies in patients with gout, and the parotid salivary carbonic anhydrase activity was studied at different flow rates because of its interest in relation to the clinical problem of dental caries and as a factor in the formation of calculus. No previous studies of uric acid and carbonic anhydrase activity in separated parotid and submandibular secretions at varying flow rates have been reported.

In all these tests the methods of saliva collection, recording the pattern of flow and the mode of application of different stimuli were carried out as described in Chapters 1 - 3.

CHAPTER 10.

HUMAN SALIVA - GENERAL REVIEW.

The salivary glands of man consist of 3 pairs of large glands (parotid, submandibular, sublingual) and the smaller glands (labial, lingual, palatal, buccal). All secrete into the oral cavity and contribute to the mixed saliva. Saliva has many important functions, none of which are essential for life. It keeps the mouth wet, facilitates speech, and lubricates food for chewing and swallowing. As it renders food substances soluble, saliva aids in the full appreciation of taste sensation. Human saliva contains the alpha-amylase pytalin which, when mixed with food by chewing, begins the digestion of starch. Its action continues as the food passes down into the body of the stomach. Saliva also secreted in response to noxious substances dilutes them and helps to cleanse the mouth. Its bicarbonate and phosphate content contribute to the buffering power of saliva, which is important in the neutralisation of acids. The rate of secretion of individual salivary glands ranges from barely perceptible during sleep (Schneyer et al 1956) to as high as 4 ml/min on maximal stimulation. Although several standard textbooks state that the total daily salivary volume is 1 - 1.5 litres the experimental evidence for these figures is lacking.

The measured daily volume of saliva in two patients with oesophageal fistulae was 500 ml (McKeown and Dunstone 1959). Jenkins (1966) suggested 620 ml. as being a more realistic estimate based partly on data of Becks and Wainwright (1943). As there is considerable variation in flow rates between individuals especially under 'resting' conditions (Becks and Wainwright 1943, Kerr 1961) there will also be a large range for the normal daily mixed saliva volume. The specific gravity of saliva varies between 1,000 and 1,010 and increased with increasing rate of flow (Kerr 1961). The osmotic pressure is between one half and three quarters that of blood (Wilsmore 1937). The viscosity of saliva depends upon the contribution of the three main glands to the saliva formed. The relative viscosities of the three main glandular secretions after acetic acid stimulation were found by Schneyer (1955) to be parotid 1.5, submandibular 3.4 and the sublingual 13.4 centipoises. He (Schneyer) points out that the viscosity is directly proportional to the percentage of mucus secreting cells in these individual glands.

Salivary Glands and Flow Rate

Most saliva is secreted by the three pairs of large glands - the parotid, the submandibular, and the sublingual. The parotid glands are serous glands which secrete fluid devoid of mucin - the submandibular glands are of mixed type containing both serous and mucous cells; the sublingual contains mainly mucous cells. The secretion of these glands

are collected in ducts whose complex histological appearance supports the conclusion based on physiological evidence, that they take an active part in the elaboration of saliva. The three pairs of glands receive a double nerve supply from the sympathetic and parasympathetic systems. The sympathetic innervation of post-ganglionic fibres come from the superior cervical ganglion to all three pairs of glands. These fibres are all adrenergic, liberating noradrenaline and they are distributed both to blood vessels and to secretory cells. Stimulation of these fibres causes vaso-constriction and scanty secretion. Pre-ganglionic parasympathetic innervation reaches the glands from the cranial outflow in the medulla oblongata by way of the glossopharyngeal and in the chorda tympani branch of the facial nerves. These fibres synapse in or close to the glands. Their post-ganglionic fibres are distributed to both secretory cells and blood vessels within the glands. Their stimulation causes vasodilation and secretion. These fibres are mediated by acetylcholine and the injection of parasympathomimetic drugs, e.g. pilocarpine, causes the same response. Atropine administration blocks the effects of acetycholine on the secretory cells, and causes a dry mouth. All salivary secretion in man, with the exception of 'paralytic' secretion is a response to nerve impulses (Davenport 1961).

In addition to the secretion produced by the three pairs of large glands, there is a contribution by the smaller glands of the oral mucosa i.e. labial, lingual, buccal and palatal mucous glands. Their relative contribution has been estimated as varying from about 30-43% (Schneyer 1955). The secretion of all glands, major and minor, differ in composition, and their relative contribution to the mixed saliva present in the mouth varies with the conditions. For example, the submandibular produces the greatest flow under 'resting' conditions. When stimulated, however, the parotid gives a proportionately higher response than the submandibular (Schneyer and Levin 1955 and Kerr 1961). The composition of the saliva produced from any one gland varies with the rate of flow which itself varies with the type and intensity of the stimulus (Dawes and Jenkins 1964).

Chemical Composition.

The composition of mixed saliva varies greatly in different individuals and in the same individual under different circumstances. For these reasons, therefore, figures for the composition of normal mixed saliva show wide ranges. Pavlov (1897) from his work in dogs reported a relationship between the nature of the stimulus and the type of saliva secreted, e.g. dry food produced a watery secretion and meat elicited a secretion rich in mucoid. It was also noted by Pickerill (1912)

that acid stimulated human saliva was alkaline. It has been widely believed since that the type and composition of saliva secreted depended upon the nature of the stimulus. Dawes and Jenkins (1964) investigated this point by studying the effects of varying flow rate on the pH and concentration of calcium, inorganic phosphate, sodium, potassium, amylase, protein nitrogen and sialic acid in separated parotid and submandibular saliva. The concentration of all these constituents was found to be dependent only on the rate of flow and not on the nature of the stimulus except for amylase. In parotid saliva at flow rates greater than 1 ml/min, the amylase concentration was higher when salt-containing stimuli were used.

No complete tables of values are available for the composition of separate salivary secretions. In Table II, 1 results derived from the data of several workers as well as some from the present studies are recorded for certain constituents of parotid saliva. It is apparent that considerable differences are present in the concentration of many salivary constituents when 'resting' and stimulated values are compared. For example sodium, chloride and bicarbonate concentrations are increased at high flow rates whereas inorganic phosphate and uric acid are reduced. The potassium concentration remains relatively constant. Systematic variation in the composition of secretion according to the rate of flow is characteristic of all digestive glands. Three general types of explanation are given (Davenport 1961) -

1) The composition of the secretion as it is extruded from the acinar cells may itself be variable.

2) The secretion as collected may be a mixture of two or more juices, each secreted at constant composition but at different rates by distinct cells. This idea explains why in the case of mixed glands such as the submandibular, samples of saliva may contain greatly differing amounts of mucin, for mucin is secreted by one cell type only, while the aqueous components are secreted by the serous cell.

   This principle is well illustrated by the gastric secretion.

3) A primary secretion from the acinar cells at constant composition may subsequently be acted upon by other cells, e.g. those of the ducts.

This latter theory of salivary secretion which postulates a primary secretion undergoing secondary equilibration across the salivary ducts is the most coherent explanation of salivary secretion so far presented. The salivary duct system is demonstrated and described in detail in Figure II, 1. The 'striated' or intralobular ducts are thought to be the most active in modifying the composition of saliva. Much evidence supports the important role of these ducts in the formation of saliva

and the following is a brief review.

The cells of the ducts are histologically much more complex than merely conducting cells need be. In certain areas they have been shown to have specific functions. Many animals studied have been found to have a duct segment specialising in water and electrolyte metabolism and structurally this segment is similar to the convoluted tubules of the kidney (Junqueira 1964). Electron microscopic studies have demonstrated plasma membrane infoldings at the base of these cells which suggest their participation in water and ion transport. Also, microvilli at the luminal surface are prominent, indicating absorptive and secretory activity (Tandler 1963).

When radioactive iodine ^{131}I is present in the blood, the cells of the ducts accumulate it and secrete iodine in high concentration into the saliva. The accumulation of ^{131}I within the cells of the ducts can be demonstrated by autoradiography (Cohen et al 1955, Logothetopoulos and Myant 1956a). The salivary gland iodide clearance, i.e. the number of mls. of blood from which iodide is removed in one minute is almost equal to the total blood flow through the whole gland (Burgen and Seeman 1957). This means that the same blood must flow past both the ducts and the acinar cells in series. There is some evidence that the blood flow is counter-current, i.e. the arterial blood passes first to capillaries

surrounding the ducts and then passes by way of a portal system to a second set of capillaries around the acinar cells (Burgen and Seeman 1958, Zimmermann 1898, and Flint 1902-3).

The diffusion gradient of sodium across the ducts is in the direction from plasma to duct and net movement of sodium occurs in that direction (Burgen and Seeman 1958). If radioactive ^{24}Na is injected into the carotid arteries supplying an actively secreting parotid gland it appears in saliva within five seconds. After subtracting the circulation time of 1-2 seconds, and the dead space time in collecting ducts of 1-2 seconds, the actual transfer time from blood to saliva is calculated to be less than three seconds. Considering the dimensions of the salivary glands, this could only be achieved in such a short time if sodium was transferred from blood to saliva without passing through the acinar cells. The simplest explanation would appear to be that sodium moves along its diffusion gradient from arterial blood into the ducts. However, it enters the ducts more slowly than the potassium leaves them. This fact suggests a tentative explanation for the hypotonicity of the parotid saliva for rapid exit of potassium and slower entrance of sodium would leave a smaller mass of osmotic material within the ducts. If the ducts are relatively impermeable to water, their contents would be hypotonic. Postulation of the relative impermeability of the ducts to water is required by any theory of parotid salivary secretion. The

fact that saliva passing through the ducts remains hypotonic has to be explained. However, it appears that the permeability of the ducts to ions and water increases as the rate of flow of saliva increases (Burgen and Seeman 1958). It would appear, therefore, that the duct cells are almost as important as the acinar cells in the transport of salivary constituents (Davenport 1961).

Several new methods have been used recently to study the actions of the duct cells more fully:-

A) The stop flow technique in which the mouth of the duct is closed and the secretion is not allowed to escape from the duct for several minutes. The secretion is then collected after release in serial fractions so that fluid in the distal, middle and proximal parts can be analysed separately, and the effect of each part of the duct can be estimated (Henriques 1961).

B) Estimation of the sodium and potassium concentration of saliva in animals has been made where the duct cells have been selectively poisoned with mercuric chloride (Henriques 1962).

C) In some animals, e.g. the rat, the acini are known to be insufficiently developed to be functional until some weeks after birth. Secretions collected before this age, therefore, represent duct fluid. By this method it has been shown that the ducts secrete at rates similar to those

of the acini per unit weight of tissue (Jacoby and Leeson 1959).

The relationship of the concentration of a salivary constituent to its concentration in the plasma is an important aspect which has been investigated mainly in animals. In dogs, changes in blood calcium have been found to cause a corresponding change in salivary calcium, whereas the injections of potassium, sodium and chloride cause little change in salivary composition. The salivary glands are therefore selective in transferring ions from the blood to the saliva. Little work on this aspect has been carried out in humans.

It has been shown in the sheep parotid gland (Denton and McDonald 1956, Coats and Wright 1957) and in man (Dreizen et al 1952, Grad 1952) that adrenocortiocosteroids and adrenocorticotrophic hormone (A.C.T.H.) cause a lowering of salivary sodium, but little change in salivary potassium. The potassium concentration of saliva is usually four to five times that of the blood plasma. The sodium concentration varies between one third and one fifteenth that of the plasma according to flow rate (Dreizen et al 1952). This would suggest that saliva is not a passive filtration from the blood, but that active processes exert a selective action, and they are influenced by adrenocorticosteroids. No other hormone effects in saliva appear to have been established.

It is apparent, therefore, that the total activity of salivary glands is extremely complex - not only with regard to specialised cellular function, but also as related to their interdependence with other organs and homeostatic systems (Tamarin 1964).

CHAPTER 11

PROBLEMS ENCOUNTERED IN ANALYSIS OF SALIVARY CONSTITUENTS

In recent years numerous studies of the salivary constituents have been made in both dental and medical disorders. There are many pitfalls in the investigations of the composition of saliva, some of which have already been mentioned in Chapters 1, 2, 3 and 10. In this chapter some of the common problems which arise in the study of the concentration of the constituents of saliva and which have been encountered in the present studies will be discussed and illustrated by examples. They will be considered under the following headings:

1: Variations in the concentration of salivary constituents with flow rate.

2: Variations in the concentration of salivary constituents with plasma levels.

3: Variations in the constituents of saliva collected from different glands.

4: Diurnal variations.

1: Variations in the concentration of salivary constituents with flow rate:

The salivary flow rate varies from patient to patient, and in one patient following various stimuli. Measurement of the flow rate is important since it is well recognised that the concentration of many of

the salivary constituents varies with flow rate. For example, the sodium and chloride concentrations rise with increase in flow rate, whereas the potassium concentration remains relatively constant (Chapter 12). On the other hand, the salivary iodide concentration decreases with increasing flow rate (Chapter 17). These facts have clinical implications, two of which will now be discussed.

A A typical set of results for sodium and potassium in parotid saliva are shown in Figure II , 2. Aldosterone has an effect on the concentration of sodium and potassium in saliva, the concentration of sodium tending to fall, and that of potassium to rise (Simpson and Tait 1955). Measurement of the sodium/potassium ratio may therefore be of value in the diagnosis of adrenal disorders including hyperaldosteronism, Cushing's syndrome and Addison's disease (Frawley and Thorn 1951), the ratio being relatively independent of dietary changes (Pawan 1955). Results must, however, be interpreted with caution if flow rate is not known. This is especially so as the largest contribution to changes in this ratio are the changes in the sodium concentration (Prunty and McSwiney, 1957). Thus Pawan (1955) has suggested that a ratio of 0.08 is characteristic of Cushing's syndrome, but as these results show, (Figure II , 2) such values may occur in normal patients at low salivary flow rates. Similar considerations apply to the measurement of salivary electrolyte concentrations in patients with fibrocystic disease in which condition increased sodium and chloride levels

have been reported (Barbero and Chernick 1958, Di Sant'Agnese et al 1958).

B Iodide is concentrated in saliva and the salivary iodide has been suggested as a possible measure of the plasma inorganic iodine (PII) concentration (Vought et al 1963), a quantity too small to be measured directly. In practice, however, this method gives poor results due mainly to the variations in salivary iodide concentration with flow rate (Chapters 17 and 26, pages 113 and 163). Similarly the ratio salivary ^{131}I/ plasma protein bound ^{131}I which has been suggested as an index of thyroid function is unsatisfactory because of the variations in the numerator due to flow rate.

The salivary flow rate can be calculated if the volume of the saliva and the duration of the collection period are recorded. This assumes that the flow rate remains constant throughout the collection period. The flow rate may vary with several factors such as application of the stimulus, and under some circumstances it may be desirable to monitor the pattern of salivary flow using a mechanical device such as the photo-electric drop recorder described in Chapter 2. Mixed saliva cannot be monitored in this way, and measurement of volume may be inaccurate due to incomplete collection.

2: Variations in the concentration of salivary constituents with plasma levels.

The relationship between the concentration of a substance in saliva and its concentration in plasma should be studied. Iodide is an example of a salivary constituent like calcium (Andreyev and Pugsley, 1933) and bicarbonate (De Beer and Wilson, 1932) whose concentration is dependent on its concentration in the plasma. Salivary levels can be interpreted, therefore, only if the plasma iodide (PII) concentration is known. As the PII rises the concentration of iodide in the saliva rises, but the saliva/plasma iodide ratio remains relatively constant. Table II, 2 illustrates this in one patient studied at four PII levels following administration of iodide supplements.

3: Variations in the constituents of saliva collected from different glands.

The composition of saliva and the volume contributed from different glands varies. One problem in using whole saliva is that the volume contributed by the individual glands varies with flow rate, over 65% of the saliva collected under 'resting' conditions being submandibular but on stimulation the parotid gives a proportionately higher response than the submandibular (Schneyer and Levin, 1955, Kerr, 1961). Accurate measurement of flow rate of mixed saliva is not therefore possible. Moreover, a dry mouth resulting from removal of the saliva is a stimulus to further saliva secretions, making basal collection difficult. The chemical composition

of parotid and submandibular saliva differs. For example, iodide, like phosphate (Dawes and Jenkins 1964) is more concentrated in parotid than in submandibular saliva. The mean iodide secretion under conditions of minimal stimulation is 0.62 µg/hr in parotid saliva, and 0.27 µg/hr in submandibular saliva (Chapter 23). On the other hand, submandibular saliva contains a higher concentration of calcium than parotid saliva (Dawes and Jenkins 1964).

It will be apparent from the above that where accurate reproducible estimations of a salivary constituent are required, pure parotid, submandibular, or sublingual saliva collections are necessary.

Saliva can be collected relatively easily from the parotid gland using a two chamber collecting device held in position over the duct orifice by air suction (Chapter 1, page 3). The secretion of the submandibular gland is, however, more difficult to obtain, and a segregator appliance may be used (Schneyer 1955) as described in Chapter 1, or a polyethylene catheter can be inserted into the duct orifice (Kerr 1961). Both methods have disadvantages as previously discussed (page 5), even when the operator is practised in the method. In certain circumstances, e.g. where the dental environment is being assessed, mixed saliva is usually collected.

4. Diurnal variations in the concentration of salivary constituents.

When saliva is to be collected at varying times of the day it is important to know whether the concentrations of the constituent under study is subject to diurnal variations. Otherwise the time of collection of the saliva should be restricted to the same time each day. Diurnal variations have been most clearly demonstrated with regard to sodium, the highest concentration occurring in the morning on rising (Grad 1954; Pawan 1955). This may be related to the diurnal variations in A.C.T.H. There is, however, no information as to possible diurnal variations in the concentration of many of the salivary constituents.

SUMMARY.

When studying and interpreting the significance of the concentration of a salivary constituent, it is usually necessary to consider:

1. The variation in the concentration of the constituent with rate of flow of saliva.
2. The relation between the concentration of the constituent in the saliva and its concentration in the plasma.
3. The method of saliva collection.
4. Possible diurnal variations.

The importance of these points has been demonstrated with regard to certain salivary constituents and in various situations where measurement of salivary constituents has been said to be of practical value.

CHAPTER 12

STUDIES ON SODIUM, POTASSIUM AND CHLORIDE IN HUMAN PAROTID SALIVA

Several thorough investigations have been carried out on the concentration of sodium, potassium and chloride in parotid and submandibular saliva at varying flow rates (Thaysen et al, 1954, Hildes 1955, Shannon and Prigmore, 1960, Dawes and Jenkins 1964). These studies in general show that the concentrations of sodium and chloride increase with salivary flow rate, whereas the potassium concentration remains relatively constant.

In the present work, preparatory base-line studies were carried out on normal subjects in which parotid salivary concentrations of sodium, potassium and chloride were estimated at varying rates of flow.

METHODS:

Ten normal subjects age range 22 to 65 years; 5 males and 5 females were studied. The subject was seated comfortably in a dental chair. 'Resting' saliva was collected for a period of 30 minutes, followed by a 5 minute sample using fruit gum stimulation and finally a 2 minute sample with lemon juice stimulation.

RESULTS

These are shown in Table II, 3. In the ten subjects the sodium concentration rose with increase in flow rate from a mean of 6.9 $\pm$ SEM 1.3 m.eq/1 at low flow rates, to a mean of 64.8 $\pm$ 3.1 m.eq/1 at high flow rates. Similarly, the chloride concentratisn rose from a mean 20.7 $\pm$ 0.9 m.eq/1 at low flow rates to a mean concentration of 32.7 $\pm$ 2.3 m.eq/1 at high flow rates. The potassium concentration however showed little change from a mean of 20.4 $\pm$ 1.1 m.eq/1 at low flow rates to 20.9 $\pm$ 0.8 m.eq/1 at high flow rates.

DISCUSSION

These results in general are similar to those previously reported by other workers (Thaysen et al 1954, Hildes 1955, Hildes and Ferguson 1955, Shannon and Prigmore 1960, and Dawes and Jenkins 1964). The sodium and chloride concentrations show a linear relationship with flow rate. Even at high flow rates their concentrations in saliva are considerably below the plasma level. In contrast the potassium concentration remains relatively constant being about five times the plasma level at all the flow rates studied. These findings cannot therefore be accounted for by a simple theory such as diffusion of the substances from the plasma and subsequent water reabsorption from the ducts. A probable explanation of these changes in concentration with varying flow rates is that the salivary ducts are not merely conducting tubes, and that the primary secretion of saliva by the acini is altered as it

passes along the salivary ducts as discussed in Chapter 10 page 66.
A diagramatic explanation from the results of Burgen and Seeman's isotope experiments in dogs is depicted in Figure II, 3, after Davenport (1961). The secretion extruded from the acinar cells at a constant composition has a high concentration of potassium and a low concentration of sodium. Subsequently changes occur across the duct cells. A large amount of potassium passes out and sodium passes into the duct lumen from the arterial blood in the adjacent capillaries. Smaller amounts of sodium pass out of the ducts and small amounts of potassium pass in. The large amounts of potassium passing into the counter-current capillary system are then circulated to the acini and this accounts for the high level of potassium secreted by the acini (Burgen and Seeman 1958). The ducts appear to be relatively impermeable to water, the content of the saliva being hypotonic. The permeability of the ducts to ions and water appears to increase as the rate of salivary flow increases. Therefore, at high rates of secretion, the sodium concentrations of saliva approach those of the plasma. Evidence has been presented that chloride and water are reabsorbed after the fluid leaves the acinar cells (Yoshimura et al 1962, Miyoshi, 1963, Henriques 1961 and 1962).

A somewhat different explanation is suggested by Chauncey and Shannon (1965). They state that sodium, potassium and chloride are probably secreted by acini after passive diffusion from the plasma. Impermeability

of the duct cells in the dog to potassium has been demonstrated (Chauncey et al 1962) but reabsorption of sodium, chloride and water occur and this explains the increased sodium and chloride levels found at higher flow rates as there is less time for reabsorption to occur. They suggest the relatively constant high potassium level at all flow rates is the resultw of active acinar transport of potassium. Shannon et al (1962) had previously suggested active secretion of potassium by duct cells. Further work is obviously necessary for a complete understanding of the mechanisms underlying the secretion of salivary electrolytes.

SUMMARY

Sodium, potassium and chloride concentrations were measured in parotid saliva of ten normal subjects, collected at varying flow rates.

The sodium and chloride concentrations were directly related to flow rate. The sodium concentration increased from a mean of 6.9 ± 1.3 m.eq/1 at low flow rates to a mean of 64.8 ± 3.1 m.eq/1 at high flow rates. Similarly, the chloride concentration rose from 20.7 ± 0.9 m.eq/1 at low flow rates to a mean of 32.7 ± 2.3 m.eq/1 at high flow rates, whereas the potassium concentration remained relatively constant.

CHAPTER 13

THE INFLUENCE OF FLOW RATE ON THE CONCENTRATION OF URIC ACID IN HUMAN PAROTID AND SUBMANDIBULAR SALIVA

Although it was suggested more than 25 years ago that estimation of the uric acid concentration in saliva might be a better index of uric acid production in the body than its concentration in blood or urine (Kallos, 1939, Maupetit 1933), few studies have been made of this constituent of saliva. One recent review contains no reference to uric acid concentration in saliva (Burgen and Emmelin 1961), and in another, it is wrongly concluded from the evidence cited that stimulation of the salivary secretion results in an increase in uric acid concentration (Afonsky 1961). The concentration of uric acid in mixed saliva has been reported as ranging from 0.5 to 20.6 mg/100 ml (Kallos 1939, Maupetit 1933, Updegraff and Lewis 1924, Morris and Jersey 1923, Sorensen 1959), and in the only study in secretions from an individual gland the concentration of uric acid in the parotid saliva collected with paraffin wax stimulation was found to range from 0.9 to 5.5 mg/100 ml. (Hawkins et al 1963).

In this chapter the results of uric acid determination carried out in parotid and submandibular saliva collected at varying flow rates will be reported.

MATERIALS AND METHODS

Six subjects (age range 38-63 years) and consisting of three males and three females were studied. They included convalescent patients who had volunteered for the studies, and members of the hospital staff. None had gout or other disturbance of uric acid metabolism. In five subjects, parotid saliva was collected and in four subjects, submandibular saliva was collected (Chapter 1). Saliva was obtained at varying flow rates, using different stimuli (Chapters 2 and 3). The graded response relative to the different stimuli employed is shown in Table II, 4. The stimuli were applied in random order. Flow rates varied from 0.05 ml/min with minimal stimulation to 2.40 ml/min after lemon juice for parotid saliva, and 0.01 ml/min to 1.40 ml/min for submandibular saliva. Each stimulus was continued until at least 2 ml of saliva had been collected. A 10 cc sample of serum was obtained in each patient.

The uric acid concentration was estimated in the saliva and serum by the standard Technicon Autoanalyser method as described in Chapter 9. Using this method, higher values were obtained compared to the uricase method, and in thirteen samples it was found that $y = 0.33 + 1.17x$ where x = value obtained using uricase method, and y = value obtained with autoanalyser method.

RESULTS

The results are shown in Figures II, 4 - II, 9 and Table II, 5. Both with parotid and submandibular saliva, the uric acid concentration was inversely related to flow rate. A straight line relation was obtained when log (vol/min) was plotted against log (uric acid concentration) at all flow rates studied.

The uric acid concentration in the parotid saliva of five patients, each studied at different flow rates, is shown in Figures II, 4, 5, 6 and 8, 9. At low flow rates of from 0.05 to 0.17 ml/min the uric acid concentration ranged from 3.1 to 5.2 mg/100 ml, mean 4.0 $\pm$ SEM 0.43 mg/100 ml (Table II, 5). As the flow rate rose, the concentration of uric acid fell to less than half the concentration at low flow rates. At high flow rates 0.75 - 2.40 ml/min, the mean uric acid concentration was 1.7 $\pm$ 0.28 mgs/100 ml. As the relation between flow rate and uric acid concentration was logarithmic, the concentration fell most rapidly over the first part of the curve.

Figures II, 4, 5 and 7, 8, show the relation between uric acid concentration and flow rate in submandibular saliva. At 'resting' flow rates of from 0.10 - 0.22 ml/min the uric acid concentration ranged from 2.1 to 4.0 mg/100 ml, mean 3.3 $\pm$ SEM 0.46 mg/100 ml. (Table II, 5). With increasing

flow rate, the uric acid concentration fell to less than half the value at low flow rates in all subjects. At high flow rates of 0.60 - 1.40 ml/min. the mean uric acid concentration was 1.3 $\pm$ 0.12 mgs/100 ml.

The correlation coefficients between log (vol/min) and log (uric acid concentration) are shown in Table 11, 6. The shape of the regression lines are not significantly different from each other.

In three subjects simultaneous collections of parotid and submandibular saliva was made. For a given flow rate, the concentration of uric acid was greater in parotid than in submandibular saliva. The absolute secretion of uric acid rose for parotid and submandibular saliva respectively from 0.27 $\pm$ SEM 0.07 mg/hr. and 0.18 $\pm$ 0.07 mg/hr at low flow rates to 0.99 $\pm$ 0.17 mg/hr and 0.78 $\pm$ 0.07 mg/hr at high flow rates (Table II, 5).

The concentration of uric acid in serum was within the normal range in all subjects studied.

DISCUSSION

The concentration of uric acid in saliva is dependent on the rate of salivary flow. Thus knowledge of the uric acid concentration of a sample of saliva is not meaningful unless the rate of flow is also known. The secretion of saliva is a complex process, the composition of saliva secreted in the acini changing as it passes along the ducts due to secretion and reabsorption of different constituents (Chapter 10 page 66). Burgen (1956b).

in a study of salivary non-electrolytes in the dog, has described two patterns of behaviour with increasing flow rate. For some constituents, e.g. N-ethylurea the concentration was inversely related to flow rate, whereas for others the relation of concentration to flow rate was U-shaped. Within the flow rates studied, the best fit for the points recorded here is a straight line when log (vol/min) is plotted against log (uric acid conc.) Uric acid, therefore, appears to belong to the first group of substances. Burgen (1956b) concluded that the relationship between concentration and flow rate in this group could be accounted for by the hypothesis that the permeability of the gland for these substances did not change significantly with alteration in the rate of flow. In his experiments, however, maximal salivary flow rate was achieved by stimulation of the auriculo-temporal nerve. The high flow rates recorded in this study may, therefore, be sub-maximal and a secondary rise in uric acid concentration might occur at higher flow rates.

The concentration of uric acid has been previously studied in mixed saliva by other workers, but little reference has been made to variations with flow. Updegraff & Lewis (1924) collected saliva during paraffin wax chewing, and found uric acid concentrations ranging from 0.5 to 2.9 mg/100 ml., values similar to those in submandibular and parotid saliva after stimulation reported in the present study.

Values ranging from 0.9 to 20.6 mg/100 ml were found by Kallos (1939) who concluded that estimation of the saliva uric acid was of value

in detecting 'a gouty tendency' and in determining the amount of uric acid formed after different types of food. Maupetit (1933) also claimed that estimation of the salivary uric acid was important clinically and Sorensen (1959) has emphasized the importance of the extra-renal excretion of uric acid.

Morris and Jersey (1923) have suggested that uric acid should be studied in 'resting' saliva because of its higher uric acid concentration. As the relation between flow rate and uric acid concentration is logarithmic, the uric acid concentration varies more markedly at low flow rates (Figures II, 4 - II, 9). Above flow rates of 1 ml/min. variations in uric acid concentration are small and precise measurement of flow rate is unimportant.

There appears to be a difference in the uric acid concentration of saliva from different glands, the concentration in submandibular saliva being less than that in parotid saliva.

SUMMARY

Uric acid concentration was measured in parotid and submandibular saliva collected at varying flow rates.

The uric acid concentration was inversely related to flow rate, and a straight line relation was obtained when log (vol/min) was plotted against log (uric acid concentration).

In parotid saliva, the mean uric acid concentration fell from 4.0 $\pm$ SEM 0.43 mgs/100 ml at low flow rates to 1.7 $\pm$ 0.28 mgs/100 ml at high flow rates. The concentrations were lower in submandibular saliva 3.3 $\pm$ 0.46 mgs/100 ml and 1.3 $\pm$ 0.12 mgs/100 ml respectively.

It is concluded that in studies of uric acid, saliva should be collected at flow rates greater than 1 ml/min, at which rates there is little variation in concentration and precise measurement of flow rate is unnecessary.

CHAPTER 14.

CARBONIC ANHYDRASE ACTIVITY IN SALIVA IN MAN.

The enzyme carbonic anhydrase is present in the salivary glands in animals (Yoshimura et al 1959; Draus et al 1962), and in man (Sand 1949). Rapp (1946) first demonstrated carbonic anhydrase activity in human saliva and suggested that the enzyme accelerated the removal of carbon dioxide, resulting in alkalinisation of the saliva. Szabo (1965) later confirmed that there was a relationship between this enzyme and salivary pH. The pH and concentration of bicarbonate in saliva have been found to increase with increasing flow rate (Anderson 1949; Thaysen et al 1954; Dawes and Jenkins 1964). It seemed of interest, therefore, to study the effect of increasing flow rate on the carbonic anhydrase activity in saliva.

The pH of saliva and carbonic anhydrase activity vary with temperature and method of collection (Szabo and Hattyasy 1965; Rapp 1946; Altschule and Lewis 1949). The effect of collection methods and of storage at different temperatures on the salivary carbonic anhydrase activity has therefore also been studied.

METHODS

Subjects Studied :

The subjects were normal volunteers, and included members of the hospital staff. Their ages ranged from 33 to 60 years, and all but one were males. None had clinical evidence of metabolic or salivary gland disorders.

Saliva Collection

Parotid saliva was collected in six subjects at varying rates of flow as described in chapters 1 - 3. In this way parotid saliva secreted at flow rates varying from 0.05 to 1.75 ml/min was obtained. In six subjects, saliva was also collected under oil. In two subjects parotid and submandibular saliva was collected after lemon juice stimulation and mixed saliva after wax stimulation on each of three consecutive days at the same time each day.

ESTIMATION OF CARBONIC ANHYDRASE ACTIVITY

The Gloster (1955) modification of the method of Krebs and Roughton (1948) was used. The estimation was conducted at a temperature of 25°C. 0.05 ml of 0.02 M.$NaHCO_3$ solution was placed in the side arm of a

Warburg Flask and O.2 ml of the saliva sample in 2.3 ml. O.1 M phosphate buffer (pH 6.98) was placed in the flask itself. In a control flask O.2 ml. distilled water was used in place of the saliva. After 10 minutes equilibration the solutions were mixed and the amount of CO_2 produced was measured at one minute intervals on the manometer. Each test was carried out in duplicate, the mean value at the fifth minute being taken as the enzyme activity. This was expressed in K/ml saliva (Mitchell et al 1945; Altschule and Lewis 1949).

In 17 samples, the enzyme activity was measured immediately after collection, and 24 and 48 hours later. Four samples were kept at $0^{o}C$, seven at room temperature and six at $37^{o}C$.

RESULTS

The carbonic anhydrase activity in saliva estimated immediately after collection and 24 and 48 hours later is shown in Tables II, 7 - II, 9. There is no significant change in enzyme activity in any sample. Furthermore, the results in the saliva collected under oil are similar to the value obtained when no oil was used (Table II, 9).

The carbonic anhydrase activity in parotid saliva increased with increasing flow rate in the five patients from resting values under 1.6 K/ml to a maximum of about 2.2 K/ml (Figures II, 10 - II, 14). As

there were no significant differences between the regression coefficients, the results from the five patients were considered together (Figures II, 15). Up to flow rates of 0.80 ml/min. there was a straight line relation between flow rate and enzyme concentration, $y = 1.001 x + 1.458$ where x = flow rate (ml/min) and y = enzyme concentration (K/ml) ($r = +0.89$, $P < 0.01$). At rates greater than 0.80 ml/min. the enzyme concentration remained constant, $y = -0.0111 x + 2.2086$. Since $r = -0.04$ which is not significantly different from 0, we can take $y = 2.1942$ as the regression of y on x. The standard deviation of y is 0.027.

The carbonic anhydrase activity in saliva remained relatively constant from day to day in parotid, mixed and submandibular saliva (Table II, 10). The enzyme activity in the blood was greater than that found in 'resting' saliva, but less than that found in saliva collected after stimulation.

DISCUSSION

In this study it has been shown that the carbonic anhydrase activity increased with flow rate to a maximum value at flow rates of about 0.75 ml/min., the concentration remaining constant at rates above this level. As described in Chapters 10 and 12 the secretion of saliva is a complex process, the composition of the precursor fluid secreted in the acini being modified in the ducts due to secretion and reabsorption of constituents. The changes in carbonic anhydrase activity with increasing

flow rate could be accounted for in a number of ways and further work is required to elucidate this problem. One explanation would be that the enzyme secreted in the acini is reabsorbed in the ducts, and that the reabsorptive process has a limited capacity. A second contributory factor might be that the permeability of the acinar and/or duct cells increases with increased glandular secretion (Burgen and Seeman 1958). If the gland were to secrete a small constant secretion free from carbonic anhydrase (v ml/min) and a variable secretion containing a constant concentration of this enzyme (K) then the relation between the concentration of carbonic anhydrase in saliva (C) and the salivary flow rate (V) would be

$$c = K - \frac{v\,K}{V}$$

However, when the observed c is plotted against the reciprocal of the observed V, the experimental data does not lie around a straight line, suggesting that the above relationship does not hold for carbonic anhydrase.

It has been suggested that the presence of carbonic anhydrase in human saliva can speed the removal of carbon dioxide from the saliva and that this results in a more alkaline saliva with an increased tendency to precipitation of calcium salts (Rapp, 1946). More recently, McConnel et al (1961) have supported the view that carbonic anhydrase plays a role in the formation of oral calculus. This enzyme accelerates the

conversion of carbon dioxide to bicarbonate, and Sand, (1951) and Wechsler (1959) have demonstrated that in addition to bicarbonate obtained from other sources, an appreciable proportion of the salivary bicarbonate is derived from metabolism in the salivary glands. Following the administration of a carbonic anhydrase inhibitor, the bicarbonate concentration in saliva decreases (Neidermeier et al, 1955). Like carbonic anhydrase, the concentration of bicarbonate in saliva and the salivary pH increase with increasing flow rate and remain relatively constant at high flow rates (Anderson, 1949, Thaysen et al, 1954, Dawes and Jenkins, 1964).

In any study of carbonic anhydrase activity in saliva it is desirable to collect the samples at flow rates greater than 0.8 ml/min at which levels the concentration appears to be independent of flow rate. Collection of the saliva under oil is unnecessary.

SUMMARY

The carbonic anhydrase activity has been measured in human parotid, submandibular and mixed saliva. In parotid saliva the activity was related to salivary flow rate, rising from less than 1.6 K/ml at low flow rates to a maximum of about 2.2 K/ml at higher rates of flow. The enzyme activity remained constant at flow rates greater than 0.8 ml/min. The concentration in submandibular saliva tended to be less than the concentration in parotid saliva. The enzyme activity in the blood was found to be 1.9 K/ml.

Enzyme activity was not affected by storage at 0^{o}C and room temperature for up to 48 hours. Similar values were found when saliva was collected under oil and when saliva was collected without oil.

PART III

THE SALIVARY GLAND IODIDE CONCENTRATING MECHANISM IN HEALTH AND DISEASE

PART III

INTRODUCTION

This part of the present work is concerned with the salivary iodide concentrating mechanism in health and disease. Experimental and clinical studies are prefaced by an outline of inorganic iodine metabolism and a review of the subject.

The physiological significance of the iodide concentrating mechanism of the salivary glands is not apparent. Even basic information such as the salivary iodide concentration in health and disease, and the absolute quantities of iodide transported by the salivary glands in unit time are not known. It is the purpose of the present work firstly to study physiological aspects of salivary iodine metabolism in man (Chapters 17, 18, 19, 20 and 21), and secondly, to study the salivary iodide concentrating ability in certain disease states. Chapter 23 deals with the effects of altered states of thyroid function and Chapter 24, with diseases affecting the salivary glands.

The effects of inhibitors on salivary iodide transport are reported in Chapter 22. Some autoradiographic animal studies concerned with the localisation of the site of iodide concentration are described in Chapter 25. As it has been suggested that the salivary iodide concentration may be used in tests of thyroid function, its use in clinical practice is

evaluated in Chapter 26. Finally, the salivary and thyroid glands are compared with special reference to their iodide concentration mechanism.

One advantage in studying the salivary gland iodide concentrating mechanism is that the saliva unlike thyroid secretions can be collected externally. Appropriate methods for collection of saliva and measurement of salivary flow rate were devised and the detailed description given to these methods in Chapters 1, 2 and 3, reflects their importance in the present study. An assessment of their efficiency was an essential preliminary to the interpretation of any quantitative data.

CHAPTER 15

OUTLINE OF INORGANIC IODINE METABOLISM

Iodine is the heaviest element normally present in biological materials. Its weight probably determines its scarcity in the earth's crust. Only minute amounts of iodine are present in plasma. The salivary and thyroid glands have made a remarkable adaptation to this dearth of iodine by having extremely efficient concentrating mechanisms. The iodine concentrated by the salivary glands is derived from the plasma inorganic iodine.

Distribution of Iodide

Following absorption from the gut iodide passes into the plasma inorganic iodine pool. Some is excreted by the kidneys and some is taken up by the thyroid, salivary, and gastric glands and small amounts by the skin and hair. In the thyroid gland iodide is converted to thyroid hormone by organification and thyroxine is secreted from the thyroidal iodine pool into the peripheral pool of organic iodine. The latter is made up of thyroid hormones in the plasma and tissues. Part of the organic iodine leaves this pool in the faeces but most is deiodinated and re-enters the plasma inorganic pool. Iodine concentrated by the salivary glands and secreted in the saliva is reabsorbed from the stomach (Brown-Grant 1961)

and small intestine (Elmer 1938) following ingestion together with iodine derived from the diet and gastric glands, (Figure III, 1).

Iodide is distributed throughout the extra-cellular fluid but most cell membranes, except red blood corpuscles (Myant et al 1950) are impermeable to iodide. The "iodide space" is a biochemical concept and is defined as the volume of fluid which would be required in order to contain the body iodide at the plasma concentration. Obviously those tissues which concentrate iodide to many times the plasma level, such as thyroid and salivary glands, will add considerably to the "iodide space".

In the fasting state the iodide concentration of the plasma (PII) varies between 0.08 and 0.60 µg/100 ml. in Scotland (Wayne et al 1964) but higher values have been reported elsewhere, e.g. North America (Table III, 1). The thyroidal iodide clearance is normally between 10 and 35 ml/min and the renal clearance ranges from 15 to 55 ml/min (Wayne et al 1964).

Availability of iodine:

Dietary iodine is mainly contained in foods and to a much smaller extent in the drinking water. Ocean water contains about 50 µgs of iodine/kilogram and seafish, as they concentrate iodine, are an especially rich source of dietary iodine.

Iodine requirements may differ widely in normal subjects as both the renal clearance and the faecal iodine excretion have a wide normal

range. It is generally agreed that the average daily requirements of iodine will be approximately 150 - 300μ gs (Nutrition Board of the National Research Council of the U.S.A.).

Iodine is rapidly absorbed from the small intestine. It appears that iodine is most easily absorbed as iodide and, although some organic iodine compounds, thyronines, may also be absorbed (Myant and Pochin 1950), free iodine and organic iodinated compounds are normally reduced to iodide during digestion and absorption. The iodinated dyes used in radiopaque contrast media as used in radiographic visualisation are, however, absorbed unchanged.

PLASMA INORGANIC IODINE

The plasma inorganic iodine (PII) is an important fundamental measurement for the understanding of iodine metabolism in health and disease (Wayne et al 1964). Unfortunately, iodide is present in such small quantities in the plasma at physiological levels that it cannot be measured directly. It can however be measured indirectly using the isotope dilution principle as described by Stanley (1949). The specific activity of the urinary iodide after a tracer dose of radio-iodine is identical with that of the plasma iodide, since the kidney cannot differentiate between radioactive and stable iodine atoms. Therefore after a tracer dose of ^{132}I—

$$\frac{^{132}\text{I Plasma}}{\text{PII}} = \frac{^{132}\text{I urine}}{\text{urinary iodide}}$$

$$\therefore \quad \text{PII} = \frac{\text{urinary iodide} \times {}^{132}\text{I plasma}}{{}^{132}\text{I urine.}}$$

Good correlation has been shown between the PII derived using this indirect method and the PII results obtained by direct measurement when the PII was sufficiently elevated (Alexander et al 1962).

An alternative method of determining the PII indirectly is to use the specific activity in the saliva after a tracer dose instead of the specific activity of the urine (Gerbaulet and Maurer 1958, Harden et al 1965a). Thus -

$$\frac{^{132}\text{I plasma}}{\text{PII}} = \frac{^{132}\text{I saliva}}{\text{Salivary iodide}}$$

$$\therefore \quad \text{PII} = \frac{\text{Salivary iodide} \times {}^{132}\text{I plasma}}{{}^{132}\text{I saliva}}$$

The results of determining the PII using salivary and urinary specific activity methods in health and disease are discussed in detail in Chapter 26 pages, 163 to 166.

The normal values of the PII described by various authors are shown in Table III, 1. The wide range of normal PII values reflects the lack of a homeostatic mechanism. Iodide is unlike sodium and chloride where the

kidneys adjust their clearance in relation to the dietary intake and so the plasma levels stay relatively constant. Although renal clearance for iodide shows no regulatory effect in man such a mechanism does exist in dogs and rats (Stanbury 1960).

CHAPTER 16

THE SALIVARY GLAND IODIDE CONCENTRATING MECHANISM - A REVIEW

The presence of iodide in saliva was first observed by Claude Bernard and reference is made to it in his lectures at the Collège de France in 1856. However, Lipschitz (1929a and b) was the first to study the saliva iodide when in 1929, following the administration of up to 20 mgs of sodium iodide/Kg intravenously, he found saliva/plasma iodide concentration ratios of 1.5 to 7.0 in dogs with parotid fistulae. Larger doses of iodide depressed the ratio until the iodide concentration in saliva approached that in the blood. Also in 1929 Barkan and Leistner demonstrated that the concentrating mechanism was 'specific for inorganic iodide' as they observed only negligible amounts of iodide in the saliva following the administration of iodinated egg-white. Lipschitz (1930) also found that massive doses of thyroxine did not increase the salivary iodide concentration beyond levels which might be expected from the metabolic degradation of thyroid hormone to inorganic iodine.

HUMAN STUDIES

The concentration of iodide in human saliva to many times the plasma level has been described by Elmer (1938) and Bruger and Member (1943).

However, accurate analytical methods for stable iodide estimations were not available at this time and subsequent quantitative results differed widely.

The use of radioisotopes of iodine for human studies with mixed saliva was first described by Schiff et al (1947) who reported salivary/plasma ratios varying from 7 to 700. Honour et al (1952) reported further radioisotopic studies on mixed and parotid saliva, lactating breast and stomach and suggested that "serous salivary glands, stomach and lactating breast possess an iodide concentrating mechanism which may be comparable to that of the thyroid". The observed high saliva/plasma ratios tended to occur with low rates of flow but there was no direct relationship between the clearance rate for iodide and the salivary flow rate. The ^{131}I was present in saliva only in the inorganic form and there was always a higher concentration of iodide in parotid than in mixed saliva. They reported a range of salivary/plasma ratios of 12 - 211. These results have since been confirmed by those of other workers also using radioisotopic methods (Freinkel and Ingbar 1953, Gabrielsen and Kretchmar 1956). All the major human salivary glands (parotid, submandibular and sublingual) concentrate iodide (Cohen & Myant 1959).

Anions which inhibit thyroidal iodide transport such as thiocyanate, perchlorate and nitrate were shown to have similar inhibitory effects on the salivary iodide concentrating mechanism (Rowlands et al 1953). The order of effectiveness (perchlorate, thiocyanate, nitrate) was shown to be the same in both glands by Edwards et al (1954) who suggested that these anions probably compete with iodide for a common transport mechanis, across the salivary cells. These anions had no effect on salivary chloride concentration (Ferguson et al 1957).

It was suggested by Thode et al (1954) that the salivary ^{131}I/plasma protein bound ^{131}I ratio 24 hours after the administration of radioiodine could be used as a test of thyroid function. Several radioistopic studies in thyroid disease states have shown that the salivary iodide trap is independent of thyroid function (Freinkel and Ingbar 1953, Gabrielsen and Kretchmar 1956, and Fellinger et al 1956). Although it has been suggested, but not substantiated, from animal studies (page108) that the salivary glands form organic iodine there is no evidence that the human salivary glands do so (Myant 1960). This is an important difference from the thyroid where iodide after accumulation participates in the iodination of tyrosine and eventually the thyroid hormones are formed. Furthermore, Myant (1960) has shown that thyrotrophic hormone of the anterior pituitary has no effect on the salivary gland iodide concentrating

mechanism whereas in the thyroid it is known to stimulate iodide concentration and induce hyperplasia. Normal saliva/plasma ^{131}I ratios have also been reported in panhypopituitarism (Freinkel and Ingbar 1953).

The chemical nature of the salivary iodine has been studied by several workers. Some (Freinkel and Ingbar 1953, Gerbaulet and Maurer 1958, and Ferguson et al 1958) have reported that the iodine in saliva is present almost entirely in the inorganic form. Others (Honour et al 1952, and Cohen 1962) using trichloracetic acid precipitation methods have demonstrated the presence of organic iodine compounds varying from 0 - 54% of the total salivary iodine concentration.

Apart from the studies in altered states of thyroid function few investigations of the iodide concentrating mechanism in disease states have been reported. External irradiation to the salivary glands results in progressive involution of firstly, the serous cells and then the mucous cells with resultant atrophy and fibrosis (Frank et al 1965). A group of patients who had received large radiation doses for cancer in the head and neck region were studied by Awwad (1959). He reported that the salivary iodide concentration power was unaffected after this treatment. The salivary iodide concentration was in fact raised because the salivary flow rates were decreased. Schneyer (1953) has reported temporarily increased

amylase concentrations in parotid saliva following therapeutic ^{131}I doses for thyroid carcinoma and subsequently an increased incidence of dental caries of unusual distribution (Schneyer & Tanchester 1954). It is of considerable interest that an inborn metabolic defect has been described in two patients who presented with hypothyroidism and were found to have absence of an iodide concentrating mechanism in thyroid, salivary and gastric glands (Stanbury & Chapman 1960, Wolff et al 1964).

COMPARATIVE STUDIES

Much information has accumulated about extrathyroidal iodide concentrating mechanisms from comparative studies in animals and some forms of plant life. Among the lower animals iodine is concentrated by the notochord and endostyle of the lamprey (Leloup 1952) and the byssus of the mussel (Roche et al 1960), and many algae (Wolff 1960) also concentrate iodine.

In the higher orders although the majority of tissues do not concentrate iodide above the level found in the plasma, a surprising number of vertebrate tissues do exhibit iodide concentrating ability (Table III, 2).

A comparative study of the salivary iodide concentrating mechanism was carried out in animals by Cohen and Myant (1959). Their findings are summarised in Table III, 3. They found a marked species variation with regard to the concentrating power of the submandibular and parotid salivary glands. A salivary iodide concentrating mechanism is present in the salivary glands of many animals and is highly developed in dogs, mice,

hamsters, guinea pigs and cotton rats, moderately in Mastomys and cats, weak in rabbits and negligible in rats.

There is considerable variation with regard to different salivary glands in the same animal and the same salivary glands in different animals e.g. the submandibular gland iodide concentrating mechanism is highly developed in the hamster and weak in the guinea pig, whereas with regard to the parotid gland, the reverse is true (Table III, 3). No specialised common type of cell peculiar to the glands in which the iodide concentrating mechanism was present has been found (Cohen and Myant 1959, Brown-Grant and Taylor 1963).

Although the salivary iodine is in the inorganic form in the majority of animals studied (Ruegamer 1953, Myant 1960), Weiss et al (1962) have reported organic iodine compounds (10-30%) in dog submandibular saliva. Fawcett and Kirkwood (1954) showed that homogenates of rat submaxillary gland in the presence of a high concentration of copper ions synthesise monoiodotyrosine (MIT) and Taurog et al (1957) have found a radioactive substance in homogenates of rat submaxillary gland incubated with radioiodide. It has been suggested by Fawcett and Kirkwood (1954) based on their in vitro observations that the salivary glands may play an important role in the peripheral metabolism of iodinated amino acids. The evidence from further experimental work does not substantiate this theory as di-iodotyrosine and thyroxine are metabolised equally well in

the presence or absence of salivary glands (Tong et al 1955, Watts 1956, Myant 1956 and 1960). No change in iodine concentrating ability has been demonstrated by the administration of thyrotrophic hormone or hypophysectomy in the mouse submaxillary gland (Taurog et al 1959) or after large doses of thyroxine in the dog (Ruegamer et al 1955). Arvy and Gabe (1950) have observed atrophy of the secreting tubules of the rat submaxillary gland after thyroidectomy.

In vitro studies have demonstrated that dinitrophenol depressed both oxygen consumption and iodide concentration in salivary and thyroid glands (Slingerland 1955, Fletcher et al 1956). Anions, thiocyanate, perchlorate and nitrate, have been shown to inhibit the concentration of ^{131}I in mouse salivary gland tissue slice experiments (Fletcher et al 1956). The order of effectiveness, perchlorate, thiocyanate, nitrate, being the same as in the in vivo human studies of Edwards et al (1954). Wolff and Maurey (1961) have compared the ability of various anions of the VIIth periodic group (bromide, cyanate, nitrate, nitrite, iodide, astatide, thiocyanate, monofluorosulfonate, selenocyanate, tetrafluoroborate, perrhenate, perchlorate and pertechnetate) to affect the iodide transport system in sheep thyroid and mouse salivary glands. Using tissue slice experiments they found a relationship between ion size, (expressed as partial molal volume), univalency, ionic shape and anion transport. They found a higher concentration occurred with pertechnetate than iodide in both

thyroid and salivary gland slices. Wolff (1964) in a review of the iodide concentrating mechanism based upon these animal tissue slice experiments, lists the following characteristics of iodide concentrating tissues:

1) Inhibition by anions SCN^-, CLO_4^-, NO_3^-

2) Concentration of other anions of VIIth periodic group.

3) Inhibition by various poisons, e.g. iodine, glycosides.

4) A requirements for potassium.

5) Half saturation with iodide near 3.10^{-5} m I^-

Some insight into the possible site of iodide concentration in salivary glands has been derived from autoradiographic studies (Cohen et al 1955, Logothetopoulos and Myant 1956 a & b) who have demonstrated marked concentration of radioiodine in relation to the ducts of salivary glands of mice and hamsters. These results did not however exclude an acinar site of transfer as well. This property of the duct cells has obtained strong support from the radioisotopic studies in the dog of Burgen et al (1959) in which they showed that after close intra-arterial injection of ^{131}I the radioiodine was detected in the saliva within a few seconds. This short time interval suggests a ductal site of transfer. By this method, Burgen and Seeman have demonstrated at least five functional areas across which selective isotope movement can occur. These areas do not correspond to any known histological characteristic of the duct cells (Burgen and Emmelin 1961). These experiments have shown

a proximal site for the ductal transfer of iodide and more distal sites for K., Na. and Cl. in that order. The increased awareness of the different workers of the important role of the ducts in the formation of saliva has already been discussed in Chapters 10 and 12 with special reference to salivary electrolytes. The possible mechanisms involved in these processes, as described by Burgen and Seeman (1957, 58), are illustrated in Figure II, 3 which is modified from Davenport (1961).

The evidence therefore from both animal and human studies suggests that the mechanism responsible for iodide concentration in salivary and thyroid glands is very similar. The movement of iodide against the electro-chemical gradient, the saturation of the mechanism by iodide, competitive inhibition by other ions and its dependence on energy producing processes (Fletcher 1956) suggests that active transport processes are involved (Brown-Grant 1961; Wolff 1964). Although, unlike the thyroid, the salivary glands are not influenced by thyrotrophic hormone (T.S.H.), it may be that the basic mechanism is still the same, but in the salivary glands it is inaccessible to T.S.H. (Myant 1960). Although much knowledge has accumulated of the extent and intricacy of the iodide concentrating mechanism, its purpose outwith the thyroid gland remains obscure.

In the patients referred to (page 107) who had congenital lack of an iodide concentrating mechanism in thyroid, salivary and gastric glands, the hypothyroidism responded well to treatment with larger doses of iodide. This suggests that when iodide is present in sufficient concentration in the plasma, diffusion can occur and compensate for lack of an iodide concentrating mechanism. However, such high plasma iodide levels are extremely uncommor and therefore an iodide concentrating mechanism is necessary for normal thyroid hormone synthesis (Wolff 1964). No known specific function has, however, been related to the extrathyroidal iodide concentrating mechanism.

CHAPTER 17

THE SALIVARY IODIDE CONCENTRATION AND FLOW RATE

The concentration of iodide in parotid and submandibular saliva was measured using chemical methods, in subjects with physiological plasma iodide levels. The effect of flow rate on the salivary iodide concentration was examined. In two patients salivary electrolytes were also measured in the same saliva samples.

METHODS

Thirty-five subjects were studied. All were convalescent patients attending the Western Infirmary and had volunteered for the studies. None had clinical evidence of salivary gland disorder or of thyroid or renal disease. Serum protein-bound iodine values were normal in all the patients. In twenty-seven patients parotid saliva was collected for 30 minutes under conditions of minimal stimulation. Either parotid duct cannulation (Kerr 1961) or the modified Carlson Crittenden cup, as described in Chapter I, was applied. In sixteen subjects submandibular saliva was collected for 30 minutes. In subjects with lower anterior teeth segregator appliances were employed, otherwise the ducts were cannulated (Chapter I).

In six subjects saliva was obtained at varying flow rates as described in Chapters 2 and 3. The graded response to the variety of stimuli

employed is shown in Table III, 4. Mean flow rates varied from 0.16 ml/min 'resting', to 1.89 ml/min after lemon juice for parotid saliva in five subjects, and 0.06 ml/min to 1.15 ml/min. for submandibular saliva in three subjects. Two ml. of saliva were collected with each stimulus, after an initial period of 15 minutes to avoid salivary 'rest transients'. (Chapter 3, page17).

In order to ascertain if the salivary iodide concentrating mechanism became fatigued, in two patients continuous 10 minute collections of parotid and submandibular saliva were obtained using a constant stimulus (fruit gums) for a period of one hour.

Laboratory estimations: These were performed for iodide, sodium, potassium and chloride as described in Chapter 9.

RESULTS

Relation between salivary iodide concentrations and flow rate

Figures III, 2 and III, 3, show the results in twenty-seven and sixteen subjects respectively, in whom saliva was collected for 30 minutes under conditions of minimal stimulation. There is a negative relation between salivary iodide concentration and rate of salivary flow for both parotid and submandibular glands.

The results of the iodide concentration in parotid saliva of three patients, each studied at different flow rates are shown in Figures II, 4,5 and 6

At low rates of flow, the salivary iodide concentration varied between 5.5 μg/100 ml and 28.5 μg/100 ml. In all three patients, as the flow rate increased up to rates between 1 and 2 ml/min. the salivary iodide concentration fell. Above flow rates of 1-2 ml/min. the curves levelled off and, despite increasing rates of flow, the salivary iodide concentration remained relatively constant. At flow rates of 1 ml/min. the salivary iodide concentration had fallen in all cases to less than 50 per cent of the resting value.

Figures III, 7 and III, 8, show the relation between the iodide concentration in submandibular saliva and flow rate. The iodide concentration in the resting saliva was about 12 μg/100 ml. The concentration fell at flow rates of 1 ml/min to 4.5 μg/100 ml in one patient and to 7.5 μg/100 ml in the other.

For comparison, in two subjects salivary electrolyte concentrations were estimated as well as iodine in the same saliva samples (Figures III, 6 and III, 8). These show similar changes with flow rate as are described in detail in Chapter 12, page 80.

Concentration of iodide in saliva collected over one hour at a constant flow rate.

Figure III, 9 shows the iodide concentration in the saliva of two patients in whom six consecutive 10-min saliva collections were made at

constant rates of flow. Fruit gums were used as the stimulus and the average rate of flow was 0.8 ml/min. There was no appreciable difference between the iodide concentrations of the various specimens suggesting that under these conditions fatigue of the iodide concentrating mechanism does not occur.

DISCUSSION

The differences in concentration with flow rate of these salivary constituents is apparent. Iodide decreases with increase in flow rate while sodium increases with flow rate and potassium remains relatively constant.

Marked changes in salivary iodide concentration occurred at different salivary flow rates. As the flow rate increased up to values of 1-2 ml/min. the salivary iodide concentration fell, both with parotid and submandibular saliva. No other similar quantitative data has been published but some confirmatory evidence is available on the shape of the curve relating flow rate to salivary iodide. Thus Ferguson et al (1956) noted a curvilinear relation between flow rate and parotid saliva/serum ^{131}I ratio, the ratio tending to become constant at rates greater than 1.0 ml/min. As mentioned previously (Chapter 5), radioisotopic studies or studies using potassium iodide supplements may give misleading results.

The changes in salivary iodide concentration which are described here at flow rates up to 1 ml/min could be explained as follows. If the

iodide pump in the ducts works at a constant rate then the concentration of iodide in saliva will be less when large amounts of saliva are secreted. This will result in a negative relationship between the salivary iodide concentration and flow rate. Evidence has been presented (page 66) which suggests that iodide is secreted via the duct cells rather than the acinar cells. This decrease in the salivary iodide concentration with increasing rates of flow is not due to fatigue of the concentrating mechanism since in each experiment the specimens were collected in a different sequence. Moreover, in the two patients in whom saliva was collected for one hour, there was no evidence of a decrease in salivary iodide concentration.

However, at rates greater than 1 ml/min. the iodide concentration remains constant. There are several possible explanations. Firstly, increased water reabsorption due to greater permeability of the duct at high flow rates (Davenport 1961) may counteract the tendency for the iodide concentration to fall. Secondly, iodide may be secreted by the acini at a constant concentration in addition to the iodide secretion mechanism in the duct. The studies of salivary ^{131}I following intra-arterial injection mentioned above (page 110), although giving information as to the most distal site at which the substance appears in the saliva, do not exclude an additional proximal site of secretion (Burgen and Emmelin 1961). At low rates of secretion an acinar iodide secreting mechanism would contribute

little to the salivary iodide, the majority being secreted via the duct cells. As the secretion rate rose, however, more of the salivary iodide would be derived from the acini and less from the ducts until at rapid rates the majority would be secreted via the acini. The saliva would then have an almost constant iodide concentration.

SUMMARY

Quantitative measurements of parotid and submandibular salivary iodide concentration have been made at flow rates varying from 0.05 to 2.6 ml/min. Salivary iodide concentration decreased as the flow rate rose. At flow rates of 1 ml/min the parotid salivary iodide concentration had fallen in all cases to less than half the resting value. At rates above 1-2 ml/min the iodide concentration did not fall further. Reasons for this pattern of secretion are discussed.

CHAPTER 18

THE SALIVARY IODIDE SECRETION AND THE PLASMA INORGANIC IODINE CONCENTRATION

The relation between the plasma inorganic iodine (PII) concentration and the salivary iodide clearance has not been studied at physiological concentrations of iodide, partly because the quantity of inorganic iodine normally found in the plasma is very small and cannot be measured directly by methods of assay now available. However, following the administration of a tracer dose of radio-iodine the PII may be derived indirectly by dividing the plasma radioactivity by the specific activity of the urinary iodide, as described in Chapter 5, page 32.

$$\text{i.e. PII} = \frac{\text{Urine I} \times \text{Plasma } {}^{132}\text{I}}{\text{Urine } {}^{132}\text{I}}$$

A significant negative correlation has been demonstrated between the PII and the thyroid iodide clearance (Wayne et al 1964). Thus subjects with a high normal PII have, on average, a correspondingly low normal thyroid clearance and vice versa. In this investigation the relation between the salivary iodide clearance and the PII has been studied in thirty-eight subjects none of whom was receiving iodide supplements.

MATERIALS AND METHODS

Thirty-eight subjects were studied, eight males and thirty females. All were volunteers and included convalescent patients and members of the hospital staff. They were clinically euthyroid and this was confirmed by radioiodine tests and estimates of the serum protein bound iodine (PBI). A tracer dose of 50 μc of ^{132}I was given orally to the subjects while fasting and the isotope dilution test was carried out as described in Chapter 5, page 30. Parotid saliva was collected in all 38 subjects. In sixteen subjects (three males and thirteen females) submandibular saliva was collected. Saliva was collected from 90 to 120 minutes after the tracer dose under 'resting' conditions. Blood was collected at 105 minutes the midpoint of the urine and saliva collections.

The plasma, urinary and salivary ^{132}I (per cent dose/ml) were measured in a well type scintillation counter. Iodine was measured chemically in the urine and saliva by a chloric acid digestion method (Farrell and Richmond 1961) as described in Chapter 9.

The plasma inorganic iodine was estimated from the specific activity in the urine. The salivary iodide clearance and the absolute amount of iodide excreted in the saliva per unit time were calculated as described in Chapter 5 page 31.

RESULTS

The PII ranged from 0.01 μg/100 ml to 0.60μg/100 ml. the upper limit of the normal range for subjects resident in the West of Scotland. The clearance of iodide in the parotid saliva ranged from 1.2 to 13.0 ml/min., mean 5.7 standard error of mean 0.50 ml/min. (Figure III, 10). As can be seen, there is no relation between the PII and the clearance of salivary iodide ($r = -0.046$, $0.8 > P > 0.7$). It follows therefore that a correlation would be expected between the PII and the absolute amount of iodide excreted in unit time. Figure III, 11 shows PII values plotted against parotid salivary iodide secretion in μg/hr. There is a significant correlation ($r = 0.66$, $P < 0.001$).

Clearance of iodide in submandibular saliva, range 0.6 to 8.8 ml/min, mean 3.4 standard error of mean 0.50 ml/min. was less than the parotid salivary iodide clearance (Figure III, 12). As with the parotid saliva, there was no significant correlation between the PII and the iodide clearance ($r = 0.16$, $0.9 > P > 0.8$). There was a significant correlation, however, between the PII and the iodide excreted in submandibular saliva in μg/hr (Figure III, 13) $r = 0.80$, $P < 0.001$.

DISCUSSION

Significant quantities of iodide are excreted by the salivary glands, the unstimulated salivary iodide clearance being of the same order as the renal and the thyroid iodide clearance. More iodide is excreted in the

parotid than in the submandibular saliva, and this confirms previous reports that the saliva/plasma radio-iodine ratio is higher in parotid than in mixed saliva (Honour et al 1952).

The relation between the plasma inorganic iodine (PII) concentration and the amount of iodide excreted in saliva has not previously been studied. In the present investigation a positive correlation has been found. Thus at high-normal PII levels the amount of iodide secreted in parotid or submandibular saliva is higher than the amount secreted at low-normal PII levels. In keeping with this is the finding that the salivary iodide clearance is independent of the PII level. While this is true, as has been shown, at physiological PII levels, the possibility remains that abnormally high PII concentrations, e.g. after iodide supplements, may be accompanied by an inverse change in the clearance rate. The evidence at present is conflicting. Thus Burgen and Seeman (1957) have shown that in dogs the saliva/plasma concentration ratio for iodide is reduced at increased PII levels. Other workers failed to demonstrate such an effect (Ferguson et al 1956).

Like the salivary iodide, the urinary iodine is derived from the PII, the renal iodide clearance being quantitatively of the same order as the salivary iodide clearance. There is, however, no specific iodide concentrating mechanism in the kidney such as is found in the salivary glands and in the thyroid. Iodide is filtered through the glomerular

membrane and part is reabsorbed in the tubules. Thiocyanate and perchlorate decrease the salivary iodide clearance (Burgen and Seeman 1957; Rowlands et al 1953; Ferguson et al 1957) but have no effect on the renal excretion of iodine (Malamos and Koutras 1962)

SUMMARY

Plasma inorganic iodine, the salivary iodide clearance and the absolute quantity of iodide secreted in saliva was measured in thirty-eight subjects, none of whom were receiving iodide supplements. The parotid salivary iodide clearance (mean 5.7 $\pm$ SEM 0.50 ml/min) was greater than the submandibular iodide clearance (mean 3.4 $\pm$ 0.50 ml/min).

The absolute amount of iodide excreted in saliva was proportional to the plasma inorganic iodine concentration (PII).

No relation was found between the PII and the parotid and submandibular salivary iodide clearance.

CHAPTER 19

SALIVA/PLASMA IODIDE RATIO

The saliva/plasma iodide ratio is a measurement often quoted in reviews of salivary gland function or iodine metabolism. Considerable variation is noted in the normal values cited by different authors. Saliva/plasma iodide ratios of 30-40 are described by Honour et al (1952) of "about 60" (Bell et al 1965) "10 or more" (Cohen and Myant 1959). Schiff et al (1947) reported a wide variation (range 7 - 700).

The saliva/plasma ^{132}I ratio is of clinical value as it has been used in the assessment of children with "athyreotic cretinism" to determine whether or not these children were in fact athyreotic or had absence of the iodide trapping mechanism (Hung et al 1962). In the former the saliva/plasma ratio is normal and in the latter the saliva/plasma ratio approaches unity (Means et al 1963).

METHODS

The studies in Chapters 18 and 19 have demonstrated the considerable variation which can occur in the salivary iodide concentration and the plasm inorganic iodine concentration. Parotid saliva/plasma ratios were measur in a group of 58 normal subjects aged 17-73 years. Saliva was collected under varying conditions, "resting", after fruit gum and lemon juice

stimulation in 21 subjects as described in Chapter 3 and under 'resting' conditions only in 37 subjects.

RESULTS

These are detailed in Figure III, 14, arranged according to flow rates. At low flow rates, i.e. below 0.25 ml/min, the mean parotid saliva/plasma ratio is 53.2 $\pm$ SEM 4.53 with a range of 11.1 to 116.4. At high flow rates, i.e. 0.70 ml/min and above, the mean parotid saliva/plasma ratio is 8.6 $\pm$ 0.68 with a range of 2.8 to 18.6.

DISCUSSION

From these results it is apparent that in normal subjects saliva/plasma ^{132}I ratios vary considerably. Values over 60 are common at low flow rates and values over 100 can occur. In contrast at high flow rates values under 10 are quite frequently found. In view of the results reported in the two previous chapters however, these results are not unexpected. One component of the ratio, the salivary iodide, has been shown to vary according to the salivary flow rate and therefore when interpreting the saliva/plasma ^{132}I ratio flow rate must be considered.

If the saliva/plasma ^{132}I ratio is measured in mixed saliva further variables will be present. Mixed saliva consists of the secretion of the parotid and submandibular glands, the sublingual, and the small labial, buccal and palatal groups of mucous glands which contribute the "residual"

secretion. In Chapter 18 it has been shown that the submandibular iodide concentration is less than the parotid in the same subject and Cohen & Myant (1959) have reported a lack of iodide concentration in human sublingual and "residual" secretion. The mixed saliva/plasma ^{132}I ratio therefore is slightly lower than in parotid saliva (Honour et al 1952).

SUMMARY

Parotid saliva/plasma ^{132}I ratios have been measured in 58 normal adult subjects, The ratios varied inversely with the salivary flow rate. At low rates of flow the mean S/p ratio was 53.2 ± 4.53 and at high flow rates the mean S/p ratio was decreased to 8.6 ± 0.68.

CHAPTER 20

VIIth PERIODIC GROUP

The affinity of the salivary and thyroid glands for iodide and various other anions of the VIIth periodic group has been studied extensively in vitro (Wolff 1964) and has already been discussed on page 109 in Chapter 16. However, there have been no studies in man and these seem desirable since the iodide trap of the salivary glands shows marked species differences (Cohen & Myant 1959). In this investigation the concentrating ability of the intact human parotid salivary gland for bromine, iodine and technetium was compared. The ions which are actually concentrated are bromide, iodide and pertechnetate.

METHODS

A solution containing a mixture of radioisotopes of three members of the VIIth periodic group (^{82}Br, ^{132}I, $^{99m}TcO_4$) was injected intravenously into each of six volunteers. The doses used were 30 μc, 50 μc and 200 μc respectively. Parotid saliva was collected at three flow rates using the methods previously described (Chapter 2). The collection of saliva was started 10 minutes after injection of the isotopes and lasted for 30 minutes under 'resting' conditions, 5 minutes during fruit gum stimulation and 2 minutes during lemon juice stimulation. Mean flow rates were 0.07 ml/min (resting), 0.49 ml/min (fruit gum stimulation) and 1.57 ml/min (lemon juice

stimulation). At the mid-point of each salivary collection a plasma sample was obtained by venepuncture. Plasma and salivary samples were counted using a nuclear Chicago automatic well-type counter. Each plasma and saliva sample was counted on three occasions. First ^{132}I plus ^{82}Br were estimated, excluding $^{99m}TcO_4$. Secondly, this count was repeated after decay of ^{132}I giving an estimate of ^{82}Br. Thirdly, the counting conditions were adjusted and ^{82}Br and $^{99m}TcO_4$ estimated. Counts for each individual isotope were obtained by solving three simultaneous equations using a Sirius computer. Salivary/plasma ratios were then calculated for each isotope at the three flow rates.

RESULTS

Salivary/plasma ratios of ^{132}I and $^{99m}TcO_4$ fall with increasing flow rate (Figure III, 15 & 16) but at all flow rates the concentrating ability for iodide was almost twice that for pertechnetate (Figure III, 17). Very low (< 3) concentration ratios for ^{82}Br were obtained.

DISCUSSION

These results contrast with the in vitro studies in animals which have suggested a greater affinity of the salivary gland for pertechnetate rather than iodide (Wolff 1964). However these in vivo studies reflect the result of all the processes preceding the delivery of saliva into the oral cavity. In vitro studies on the other hand represent the activity of the iodide trap in

the very artificial conditions in which salivary gland slices are studied. Wolff's results in the mouse salivary glands in vitro are therefore not inconsistent with these in vivo results in human parotid saliva.

The very low concentration of bromine is in keeping with its behaviour in Wolff's in vitro experiments. Since the knowledge that members of the VIIth periodic group are competitive inhibitors of iodide transport various attempts have been made to find that particular property which has established their common effect. It appears that univalency, anion size, and anion shape are possible factors (Wolff and Maurey 1961).

CHAPTER 21

THE CHEMICAL NATURE OF THE SALIVARY IODINE

The evidence on the chemical nature of the salivary iodine is conflicting. Some workers (Freinkel and Ingbar 1953; Ruegamer et al 1955; Gerbaulet and Maurer 1958; and Ferguson et al 1958) have reported that the iodine in saliva is present almost entirely in the inorganic form. However, in one of these observations (Freinkel & Ingbar 1953) only a short interval (2 - 3 hours) elapsed after the administration of a tracer dose of ^{131}I and others were made not in man but in animals (Ruegamer et al 1955). Evidence of a species and individual salivary gland variation is provided by the work of Weiss et al (1962). Using trichloracetic acid precipitation (TCA) technique, they have demonstrated the formation of organic iodine compounds (commonly from 10 - 30 per cent) in the parotid saliva but not in submandibular saliva of the dog. Using similar methods they failed to demonstrate organic iodine in parotid or mixed saliva of man. Honour et al (1952) using TCA technique in man observed that while in most cases the unprecipitated fraction contained nearly all the radioiodine, in a few cases the precipitable radioactive iodine accounted for almost half the total. Cohen (1962) also using TCA precipitation in man found that 24 hours after a tracer dose of radioiodine from 0 to 54 per cent of the radioactivity in mixed saliva was present in the precipitable

fraction.

The chemical nature of the salivary iodine has clinical implications. The plasma inorganic iodine, an important investigation in the diagnosis and treatment of thyroid disease, is too small to be measured directly. It can, however, be derived indirectly using either the specific activity, after a tracer dose, of the saliva or the urine. (Chapter 5, page 32). Knowledge, therefore, of the chemical nature of the salivary iodine in normal and disease states is necessary in order to assess the suitability of the saliva for this clinical test. The clinical applications are discussed in Chapter 26, page 163.

There are, however, two main difficulties to the solution of this problem. Firstly, direct chemical measurement of organic iodine compounds would be inaccurate at the low concentrations studied. Secondly, using radioistopic methods in man it is necessary to wait for at least 72 hours to assess if organification has occurred. There is therefore insufficient time for equilibration and due to the decay of the permitted radiation dosage in normal subjects, there may be so little radioactivity that accurate results cannot be obtained after 72 hours. It was, however, possible to examine the saliva of thyrotoxic and thyroid carcinoma patients after they had received larger doses of radioiodine.

The following studies on the chemical nature of the salivary iodine will be described:

a) DIRECT EVIDENCE

b) INDIRECT EVIDENCE

a) DIRECT EVIDENCE:

Methods.

In 9 thyrotoxic patients samples of blood and saliva were collected four days after a tracer dose of 150 μc of ^{131}I, and in 14 patients after a therapeutic dose of radioiodine. In this study isotopic equilibrium was not of course reached, and a proportion of the circulating organic compounds may have been due to the therapeutic dose of ^{131}I in some patients (Stanbury and Jannsen 1963). The radioactivity of plasma and saliva was measured before and after passage through an anion exchange column (Amberlite IRA-400Cl). Trichloracetic acid (TCA) (10%) was added to aliquots (4:1) of the same samples. The precipitate was washed twice with TCA, the volume of the final precipitate was corrected with deionised water to 1 ml. and the radioactivity measured. Ascending paper chromatography was performed on untreated samples of saliva using BuOH: HAc: H_2O (78: 10: 12) as solvent. The strips were scanned using a Nuclear Chicago Actigraph II. After autoradiograms of the same strips had been prepared the strips were stained with ceric arsenite reagents.

In a further two patients one with thyroid carcinoma and euthyroid, the other with thyrotoxicosis, parotid and mixed saliva samples were collected four days after a 100 mc therapeutic dose of ^{132}I. The radioactivity was measured before and after passage through the Amberlite column.

RESULTS

Thyrotoxic patients: (receiving 150 μc dose). The percentage of the total radioactivity in saliva after Amberlite resin was considerably lower than the percentage precipitated by TCA (Table III, 5). With Amberlite resin, mixed saliva was found to have less than 5 per cent organic iodine, and parotid and submandibular saliva, less than 0.5 per cent organic iodine. The discrepancies between these results and the values of 0.4 to 24.7 per cent for mixed saliva, 1.7 to 23.2 per cent for parotid saliva, and 5.4 per cent for submandibular saliva found after TCA precipitation are discussed below. Radiochromatography and autoradiography of untreated samples of saliva show only an iodide peak (Figures III, 18-20) in parotid and mixed saliva.

High Dosage (100 mc) patients: The percentage of the total radioactivity after Amberlite resin was 0.87 and 1.49 in parotid saliva and 6.64 and

1.77 in mixed saliva respectively in the two patients studied (Table III, 6). Radiochromatography revealed only an iodide peak as in the thyrotoxic patients.

DISCUSSION

Cohen (1962) using T.C.A. precipitation noted that 24 hours after a tracer dose of radioiodine from 0 to 54 per cent of the radioactivity in the saliva was present in the precipitable fraction. The results presented here show an obvious discrepancy between the values obtained using a resin and those using T.C.A. precipitation. Radiochromatography revealed only inorganic iodine and this confirmed the Amberlite resin results. One possible explanation of this discrepancy is that the values obtained after T.C.A. are falsely high as a result of some iodide precipitation with the salivary proteins. It was observed that when serum bovine albumin was added to the samples the radioactivity in the precipitable fraction increased considerably. This effect on the addition of albumin has been noted previously by Weiss et al (1962).

Because of the high therapeutic dose (100 mc) of ^{131}I used in the two patients one with thyroid carcinoma and the other thyrotoxicosis optimal conditions for accurate measurement of radioactivity are provided. Only small quantities of organic iodine compounds 2% are present in both parotids and one sample of mixed saliva from the euthyroid patient. The

mixed saliva from the thyrotoxic patient however revealed 6.64% organic iodine.

b) INDIRECT EVIDENCE

In the urine in normal subjects iodine is almost entirely in the inorganic form (Pitt-Rivers and Tata 1959) and the excretion of organic iodine compounds can be safely disregarded in normal subjects (Riggs 1952). As the body cannot distinguish between isotopes of iodine and stable iodine both follow the same metabolic paths. After a tracer dose of radioiodine the specific activities of the salivary, urinary, and plasma iodide are all equal, i.e.

$$\frac{^{132}\text{I saliva}}{^{127}\text{I saliva}} = \frac{^{132}\text{I urine}}{^{127}\text{I urine}} = \frac{^{132}\text{I plasma}}{^{127}\text{I plasma}}$$

As the PII (^{127}I plasma) is too small to measure directly this isotope dilution principle is used to derive the PII indirectly from measurements of the saliva or urine specific activity, as described in Chapter 5, page 30 These methods depend upon the assumption that all the salivary or urinary iodine is derived from the PII and not from organic compounds. Using the above equation the salivary stable iodine can be calculated, viz.

$$^{127}\text{I saliva} = \frac{^{127}\text{I urine} \times ^{132}\text{I saliva}}{^{132}\text{I urine}}$$

If we assume therefore from the previous evidence cited that the urinary iodine is in inorganic form, the calculated value for the salivary stable

iodine should correlate with the estimated concentration of the salivary iodide using chemical methods if the salivary iodine is in inorganic form.

METHODS

The isotope dilution test as described in Chapter 5, pages 30 and 31 was used in 27 subjects who had no evidence of salivary or thyroid gland disease. From the results of salivary, urinary stable iodine and radioisotopic iodine estimations the predicted value of salivary iodide was calculated from the equation previously described. The salivary iodide was measured chemically (as described in Chapter 9, page51).

RESULTS

These are shown for 27 subjects in Table III, 7. The mean parotid salivary iodine (μg/100 ml) by calculation is 6.70 ± 1.33 and by chemical measurement is 6.93 ± 1.51. In 9 of these subjects the mean submandibular salivary iodine (μg/100 ml) was, by calculation 5.39 ± 1.00 and by chemical measurement 5.51 ± 0.95. There is no significant difference between the predicted and observed values (parotid saliva $0.5 > P > 0.4$; submandibular $0.5 > P > 0.4$)

CONCLUSIONS

The studies reported on the chemical nature of the salivary iodide would indicate that the iodine in saliva is almost entirely in the inorganic form. In mixed saliva of one subject, a thyrotoxic patient who had

received a large therapeutic dose of radio-iodine, the organic iodine fraction was greater than 5%.

The discrepancies between the high values using T.C.A. precipitation and Amberlite resin were confirmed in the present studies. The former could be falsely high because of some iodide precipitation with the salivary proteins.

The agreement between the predicted and observed values for the salivary iodide using the specific activity principle adds some confirmatory evidence to the direct estimations reported. If a significant percentage of the salivary iodide was organic one would have expected the observed value to be higher than the value obtained indirectly from the specific activity of the urinary iodine.

CHAPTER 22

INHIBITORS

Various anions, e.g. perchlorate, thiocyanate and nitrate block the trapping of iodide in both the salivary and thyroid glands (Wolff 1964). The salivary/plasma iodide ratio falls after the administration of these compounds (Rowlands et al 1953; Edwards et al 1954; Burgen and Seeman 1957; Ferguson et al 1957; Mason et al 1966a).

In this chapter the effects of potassium perchlorate on the salivary iodide and salivary electrolytes was studied. Also the effect of perchlorate on the saliva/plasma ratios of pertechnetate, iodine and bromine. Its action is compared with that of carbimazole an anti-thyroid compound which blocks the synthesis of thyroid hormones after iodide trapping has occurred.

METHODS

Experiment A: In two volunteer subjects saliva was collected at varying flow rates 1 hour after the administration of 500 mg. potassium perchlorate orally. In one subject parotid saliva and in another submandibular saliva was collected.

Experiment B: In one volunteer the concentration of iodine in saliva was estimated at five minute intervals following the oral administration of potassium perchlorate 500 mg.

Experiment C: In two volunteers using the methods described previously, pages 127 and 128 the saliva/plasma ratios of iodide, technetium and bromine were studied before and 30 minutes after the oral administration of 500 mg potassium perchlorate. In one subject parotid saliva and in another subject, submandibular saliva was collected at three different flow rates.

Experiment D: In eight thyrotoxic patients the saliva/plasma iodide ratios were measured before and after the administration of carbimazole 30 mg /day for two weeks.

RESULTS

Experiment A: Figures III, 21 and III, 22 show the iodide concentration in saliva at different flow rates one hour after administration of 500 mgm of potassium perchlorate orally. The blocking effect of this drug on the salivary iodide trap can be clearly seen. In contrast the patterns of sodium, potassium and chloride secretion remain unaltered (Figure III, 22). In one subject the secretion of iodide in parotid saliva fell from 14 μg/100 ml to 2.5 μg/100 ml and in another the iodide in submandibular saliva fell from 12 μg/100 ml to 0.2 μg/100 ml.

Experiment B: Figure III, 23 shows the serial salivary iodide measurement every five minutes following the oral administration of 500 mg of potassium perchlorate. The iodide concentration falls from 2.0 μg/100 ml

to 0.1 μg/100 ml in 25 minutes and from then varies from 0 - 0.4 μ g/100 ml up to 90 minutes.

Experiment C: Tables III, 8 and III, 9 show the marked fall in saliva/plasma ratios of technetium, iodine and bromine after perchlorate administration. The flow rates before and after administration are similar and permit comparisons to be made.

The parotid 'resting' saliva/plasma ^{132}I ratio falls from 66.30 to 10.00 after perchlorate and at high flow rates from 14.80 to 1.90 (Table III, 8). For technetium comparable values are 39.50 and 3.79 for 'resting' and 7.77 and 0.68 at high flow rates, and for bromine 1.65 reduced to 0.17 and 0.79 to 0.29.

The submandibular 'resting' saliva/plasma ^{132}I ratio falls from 23.00 to 8.90 and at higher flow rates from 7.30 to 1.83 (Table III, 9). For technetium comparable values are 9.45 and 1.86 for 'resting' and 3.50 and 0.45 at higher flow rates, and for bromine 1.09 reduced to 0.26 and 1.12 to 0.58.

Experiment D: Figure III, 24 shows the parotid saliva/plasma ^{132}I ratio before and following the administration of carbimazole 30 mg daily (10 mg t.i.d.) No significant decrease in the ratio occurs. Prior to carbimazole the saliva/plasma ratio was 34.6 ± 3.17 and following carbimazole 38.3 ± 2.03.

DISCUSSION:

The results for iodine described here are similar to those of Ferguson et al (1957) who noted after potassium perchlorate a decrease in the salivary/plasma ^{131}I ratio but no change in the chloride excretion. In both the thyroid and salivary glands iodide is inhibited by perchlorate, thiocyanate and nitrate, and Edwards et al (1954) have shown that the order of effectiveness of these anions is the same in salivary glands and in the thyroid. They have suggested that in both salivary and thyroid glands, the inhibition is the result of competition between iodide and the inhibiting anion for a common transport process. Further evidence for this view is that thiocyanate (Crandall and Anderson 1934) and perchlorate (Edwards et al 1954) are concentrated by salivary glands and thiocyanate (Logothetopoulos and Myant 1956b) and perchlorate (Wolff 1964) are concentrated by thyroid glands.

The results of experiment 'C' confirm the in vitro tissue slice experiment findings of Wolff (1964) except in so far as complete inhibition of iodine, technetium, and bromine concentration does not occur. A possible explanation could be that the test starting 30 minutes after the perchlorate had been given orally, its effect was not complete. However, a maximal effect should have occurred one hour afterwards i.e. when the high flow rate values were obtained. Rowlands et al 1953

also found lack of complete inhibition of the salivary iodide trap using a similar dosage. They reported reductions to about one seventh of the original value at one hour. The reason for this difference will require further investigation. A further experiment, similar to experiment 'C', has since been carried out in which potassium perchlorate (500 mg) was given one hour before intravenous injection of iodide and technetium and a similar lack of complete inhibition was found. It is of interest that after perchlorate the residual iodide secretion is also dependent on flow rate but even at high flow rates concentration still occurs. These results suggest that at least two mechanisms are involved in salivary iodide concentration.

SUMMARY

The salivary concentrations of iodide, pertechnetate and bromide are depressed by potassium perchlorate but not completely inhibited.

The mechanism of the residual concentration of these ions is uncertain and is at present being investigated. However, the results suggest that at least two mechanisms are involved in salivary iodide concentration.

The saliva/plasma ^{132}I ratio is unaffected by carbimazole administration.

CHAPTER 23

SALIVARY IODIDE IN THYROID DISEASE

The close similarity between the salivary and thyroid iodide traps has already been described in Chapter 16 and will not be reiterated here. Thode et al (1954) have found a correlation between the radioiodine secreted by the salivary glands 24 hours after the administration of a tracer dose and thyroid function. On the other hand, other workers (Freinkel and Ingbar 1953; Fellinger et al 1956; Gabrielsen and Kretchmar 1956; and Ferguson et al 1957) have found that the saliva/plasma radioiodine ratio was not consistently altered by variations in thyroid function. However, the results of isotope experiments must be interpreted with caution. For example, it has been shown that the measurement of the thyroidal radioiodine uptake and the measurement of the protein bound radioiodine in the plasma may be misleading and not related to the absolute quantities of stable iodine taken up by the gland or the amount of hormone produced (Koutras et al 1961). Similarly, the absolute quantity of iodine secreted in the saliva cannot be estimated without chemical iodide measurements. Quantities of iodide secreted in saliva were measured and compared with the amount of iodide taken up by the thyroid gland in euthyroid, hypothyroid and thyrotoxic patients.

MATERIALS AND METHODS:

Patients studied: Fifty-seven patients were studied. Twenty-one, 16 females and 5 males were members of the staff or volunteers and were euthyroid. Twenty-four patients, 19 females and 5 males were thyrotoxic and 12 patients, all females were hypothyroid. Their thyroid status was assessed clinically using the criteria of Wayne (1960) and the diagnosis was confirmed by radioiodine tests and estimation of the serum protein-bound iodine (PBI).

Procedure: A tracer dose of 50 μc of ^{132}I was given to all the patients, fasting, along with a glass of water to ensure adequate hydration. The radioiodine uptake of the thyroid gland was measured at 60 and 150 min. using a directional scintillation counter (Alexander et al 1962).

After the first uptake measurement the patient was seated comfortably in a dental chair and the mouth was rinsed with an iodine-free mouth wash. The collection methods described in Chapter 1 were used to obtain both parotid and submandibular saliva in 8 euthyroid subjects, 16 thyrotoxic and 9 hypothyroid patients. In the remainder, parotid saliva alone was collected. A few drops of lemon juice were dropped on the tongue to ensure an adequate initial flow of saliva and the

saliva, collected during the subsequent period (10 - 15 mins.) until 'resting' levels were re-established, was discarded. A 30 min. saliva collection was then made commencing 90 min. after administration of the tracer dose, under 'resting' conditions. The routine isotope dilution test described in Chapter 5 was carried out. (Pages 30-33).

The thyroid clearance of iodine and the absolute uptake of stable iodine by the thyroid gland (AIU) were estimated as described by Wayne et al (1964) using the formula:

$$\text{Thyroid clearance} = \frac{150 \text{ min } {}^{132}\text{I uptake} - 60 \text{ min } {}^{132}\text{I uptake}}{{}^{132}\text{I plasma} \times \text{time between 2 uptakes}}$$

and

$$\text{AIU} = \text{PII} \times \text{Thyroid clearance.}$$

RESULTS:

The results are summarized in Tables III, 10 and III, 11.

Euthyroid subjects: The serum PBI and the plasma inorganic iodine (PII) concentrations and the absolute iodine uptake by the thyroid gland (AIU) are similar to the values previously reported in a control group (Wayne et al 1964). More iodide was secreted in the parotid saliva (mean 0.62 $\pm$ SEM 0.115 μg/hr) than in submandibular saliva (0.27 $\pm$ 0.051 μg/hr). The mean iodide secretion in the saliva from the two parotid and submandibular glands under basal conditions, 1.8 μg/hr, is of the same order as the amount taken up by the thyroid, 2.1 μg/hr.

Thyrotoxic subjects: In the 24 thyrotoxic patients the serum PBI ranged from 8.1 to 19.1 μg/100 ml; mean 12.4 $\pm$ SEM, 0.53 μg/100 ml. Although the PII was lower in the thyrotoxic than in the euthyroid patients, the AIU, 19.6 $\pm$ 2.68 μg/hr, was significantly higher than in the euthyroid group, 2.1 $\pm$ 0.36 μg/hr ($P < 0.001$). If only the patients in whom the submandibular saliva was collected are considered, the difference in the PII is less marked, 0.10 μg/100 ml in the thyrotoxic patients and 0.14 μg/100 ml in the euthyroid patients. The parotid and submandibular salivary flow rates were not significantly different from the rates in the euthyroid patients. There was no evidence in the thyrotoxic patients of an increase in the salivary iodide secretion paralleling the increase in the absolute iodine uptake by the thyroid. Indeed, the iodide secreted in the parotid saliva 0.31 $\pm$ 0.071 μg/hr, was significantly less than the iodide secreted in the parotid saliva of the euthyroid patients, 0.62 $\pm$ 0.115 μg/hr ($P < 0.025$). There was no significant difference in the amounts of iodide secreted in the submandibular saliva when the euthyroid and the thyrotoxic patients were compared. 0.27 $\pm$ 0.051 and 0.28 $\pm$ 0.064 μg/hr respectively.

Hypothyroid subjects: In the 12 hypothyroid patients the serum PBI and the AIU were significantly lower than in the control group ($P < 0.001$ and $P < 0.05$, respectively). However, the parotid and submandibular salivary flow rates and the iodide secreted in the parotid saliva, 0.61 $\pm$ 0.103 μg/hr,

and the submandibular saliva 0.47 $\pm$ 0.146 µg/hr, were not significantly different from the values obtained in the control patients.

DISCUSSION:

Although variations in the absolute amounts of iodine taken up by the thyroid gland are as much as 100-fold when euthyroid, hypothyroid and thyrotoxic patients are compared, no such differences are found in the amount of iodide excreted in the saliva. The concentrating ability of the parotid and submandibular glands for iodide appears to be independent of thyroid function. Other workers have studied the gradient of radioiodine in saliva and plasma, but not the absolute amount of stable iodine excreted, and have suggested that the salivary iodide concentrating mechanism is unrelated to thyroid function (Freinkel and Ingbar 1953; Fellinger et al 1956; Gabrielsen and Kretchmar 1956; Stein et al 1957).

In dogs too, Ruegamer et al (1955) found that the amount of iodide excreted in the saliva was not influenced by exogenous thyroid hormone, but was a function of the circulating plasma iodide. Furthermore, alterations in thyroid function following hypophysectomy or TSH administration does not result, in the mouse, in any change in the submandibular iodide pump (Taurog et al 1959).

Nevertheless, Thode et al (1954) found that the ratio of the saliva radioiodine to the plasma protein-bound radioiodine was low in hyperthyroidism relationship between salivary and thyroid gland function. They suggested that this ratio might be used as a diagnostic test of thyroid function. The fallacies of their deductions have been reviewed by Fellinger et al (1956). In hyperthyroidism the thyroidal uptake of radioiodine is high and therefore the plasma radioactive iodine levels fall rapidly. In hypothyroidism the reverse is true, the plasma iodide levels fall rapidly. In hypothyroidism the reverse is true, the plasma iodide level falling more slowly. As the concentration of iodide in the saliva is dependent on the inorganic iodide level in the blood (Ferguson et al 1956) the salivary radioiodine is therefore low in thyrotoxicosis and high in hypothyroidism. This difference is accentuated by dividing the salivary radioiodine by the plasma protein-bound radioiodine, the latter being high in thyrotoxicosis. Thus the ratio of the saliva radioiodine to the plasma protein-bound radioiodine is merely an index of the amount of inorganic circulatory iodide relative to the organic circulating iodine.

Differences in the salivary flow rates and in the levels of inorganic iodide in the plasma (PII) can explain the slight differences which have been found in the secretion of euthyroid, thyrotoxic and hypothyroid patients. In the thyrotoxic patients in whom parotid saliva was collected, the PII,

0:09 μ g/100 ml. was less than the value in the euthyroid patients, 0.19 μ g/100 ml. Other workers have also found a low PII in thyrotoxicosis (Aboul-Khair and Crooks 1965). As the concentration of iodide in the saliva is dependent on the level in the plasma, a low plasma level of iodide would result in a low salivary level.

Honour et al (1952) studied the secretion of radioiodine in the saliva after an intravenous injection of ^{131}I and found the saliva/plasma radioiodine concentration always higher in parotid juice than in mixed saliva. The present finding that the parotid gland secretes more stable iodide in the saliva when compared to the submandibular gland is in agreement.

Although the iodide concentrating mechanism of the salivary gland is independent of thyroid function, consideration of this trap is important in any study of iodine kinetics. Quantitatively, the amount of iodide secreted in the saliva of the two parotid and submandibular glands in unit time is, in euthyroid subjects, of the same order as the amount taken up by the thyroid gland. However, iodide secreted in saliva is not lost from the body as it is rapidly reabsorbed after passing into the small intestine.

SUMMARY:

The amount of iodide secreted in the parotid and submandibular saliva in unit time has been compared to the amount taken up by the thyroid gland (absolute iodine uptake or AIU) in 21 euthyroid, 24 thyrotoxic and 12 hypothyroid patients. Although the AIU was found to be high in thyrotoxicosis and low in hypothyroidism, no such differences were found in the salivary iodide secreted. It is concluded that the iodide concentrating mechanism in the salivary glands is independent of thyroid function. More iodide is excreted in parotid than in the submandibular saliva. In euthyroid patients almost as much iodide is secreted in the saliva as is taken up by the thyroid gland.

(1) SJÖGREN'S SYNDROME

METHODS:

The clinical details of the 15 patients studied are shown in Table III, 12, three patients were male and 12 female, the ages ranging from 47-78 years. All complained of xerostomia and had evidence of kerato-conjunctivitis sicca. The latter was confirmed by the diminished wetting of a filter paper strip during a five minute Schirmer test for tear secretion rose bengal staining of the conjunctiva and the finding of filamentary or punctate keratitis on slit lamp examination (Bloch et al 1965). Five patients had rheumatoid arthritis diagnosed on the basis of the American Rheumatism Association criteria (Ropes et al 1958). A summary of the laboratory investigations is included in Table III, 13.

STUDIES OF SALIVARY GLAND FUNCTION

(a) Sialography: A hydrostatic technique was employed using sodium metrizoate (Triosil '45) as the contrast medium (as described in Chapter 4).

(b) Measurement of Salivary Flow Rate: Parotid saliva was collected using a modified Carlson-Crittenden cup or by cannulation of the parotid ducts (Kerr 1961) as described in Chapter 1. The saliva was passed via polyethylene tubing through a drip chamber and each drop recorded using the photoelectric detector system described in Chapter 2. Saliva was

C H A P T E R 24

THE SALIVARY IODIDE IN SALIVARY GLAND DISEASE

Evidence that the salivary gland iodide concentrating ability is completely or partially a function of the duct epithelium in animal and human experiments is advanced in Chapters 16, 17 and 25. Although there is no direct evidence that this is the site of iodide concentration in man it was felt that it would be of interest to examine the iodide concentrating power of the human salivary glands in disease states. Two diseases involving salivary glands were studied. 1) Sjögren's disease in which there is loss of the secretory parenchymal cells with infiltration of lymphocytes and plasma cells, proliferative changes affecting the cells lining the ducts, and diminished or absent salivary secretion (other aspects of this condition are considered in detail in Part IV), and 2) Fibrocystic Disease, where the exocrine gland function, in particular, the pancreas, salivary, sweat and bronchial glands are affected. From studies of parotid saliva in patients with fibrocystic disease, it has been shown that the concentration of sodium, calcium and phosphorus are increased (Chauncey et al 1962) and Bessman (1956) has reported high iodide values. It has been suggested that some abnormality of salivary duct function occurs in this condition (Chauncey et al 1962; Mandel et al 1965).

collected under 'resting' conditions and after fruit gum, lemon juice (Chapter 3), and occasionally paraffin wax stimulation was used. The collections were continued until 1 ml. of saliva was obtained at each flow rate but this was not always possible especially under 'resting' conditions.

(c) Measurement of Salivary/Plasma ^{132}I Ratio: This was carried out as detailed in Chapter 5, pages 30 and 32.

RESULTS (Table III, 14).

Of the 15 patients studied 5 had normal sialograms and 10 abnormal. Under 'resting' conditions no saliva was collected in 8 patients. After stimulation with fruit gums and lemon juice salivary flow rate was abnormally low in 8 patients and all of these had abnormal sialograms. In 3 of these patients no saliva was collected even after maximal stimulation with lemon juice. All of the 5 patients with normal sialograms had normal salivary flow rates.

The saliva/plasma ^{132}I ratios in those patients in whom it was possible to collect saliva are shown in Table III, 14. Firstly in all 5 patients with normal sialograms the ratios lay within the range found in normal subjects (Figure III, 14). In 4 of the patients with abnormal sialograms the saliva/plasma ^{132}I ratio was low for the flow rates studied. In another 3 patients with abnormal sialograms the ratios were found to be normal.

DISCUSSION

Oral symptoms are a common manifestation of Sjögren's syndrome and more detailed studies of oral aspects of this condition are reported in Part IV.

It is well recognised that in this condition there are alterations in the gland parenchyma and in the ducts (Morgan and Castleman 1953; Bloch et al 1965). Indeed Morgan and Castleman have described obliterative hyperplasia and epi-myoepithelial transformation of intra-lobular ducts as pathognomonic of Sjögren's syndrome. Bloch et al (1965) however, found a wide variation in the histological pattern and in only 40% of patients were epi-myoepithelial islands found. These variations of duct involvement may account for the saliva/plasma ^{132}I ratios found in the present study as it is generally believed that the site of the iodide concentrating mechanism is probably in the duct cells of the salivary glands. It is of interest, therefore, that in 4 of the 7 patients with abnormal sialograms in whom saliva could be obtained, low saliva/plasma ratios were obtained and that these 4 patients had in fact a more severe degree of sialectasis compared with the remaining three patients in whom sialographic changes were minimal.

(2) FIBROCYSTIC DISEASE

METHODS

In 9 patients with established Fibrocystic disease confirmed by laboratory tests and electrolyte changes in the sweat, an oral tracer dose of 25 μc ^{132}I was given and the routine isotope dilution test carried out. Parotid saliva was obtained using the Carlson-Crittenden cup and one

stimulus, fruit gums, was applied. The patients' ages ranged from 6 - 12 years of age.

RESULTS:

These are recorded in Table III, 15. All the plasma inorganic iodine (PII) and salivary iodide (SI) concentrations fall within the normal adult range (no figures for children are available) except in one subject 9a, where both the salivary iodide and plasma inorganic iodine concentrations are raised. The saliva/plasma ratio is, however, within the adult normal range and the high salivary iodide is in fact the result of the high plasma level. In this boy the high salivary iodide concentration was caused by increased ingestion of iodide probably contained in a cough mixture. Further examination three weeks later, 9b, showed that these abnormally high values had returned to normal.

DISCUSSION:

Examination of the saliva provides a convenient means of studying possible changes in exocrine gland function as in patients with cystic fibrosis. Submandibular saliva was not examined because of the difficulti inherent in collecting submandibular saliva in these young children. All the previous work suggesting a salivary duct defect in this condition has been carried out in parotid saliva. Elevations of sodium and chloride concentrations in the saliva have been reported (Di Sant'Agnese 1958,

Barbero and Chernick 1958). However, in a carefully controlled study Chauncey et al (1962) reported that such changes were of only borderline significance. Salivary calcium (Chauncey et al 1962) and inorganic phosphorus (Chauncey et al 1962, Mandel et al 1965) were found to be elevated in patients with cystic fibrosis and it has been suggested that a defect exists in the parotid gland duct system. Bessman (1956) reported, after administration of iodine supplements to normal children and others with fibrocystic disease, abnormally high salivary iodide values. The results reported here show no elevation of saliva/plasma ^{132}I ratios in the patients studied as compared with the normal adult range. No normal range for children is available and a strict comparison would require the use of radio-isotopic methods in normal children which is not justifiable. These findings reported here, however, demonstrate the importance of relating the salivary iodide concentration to the plasma inorganic iodide level. The latter varying considerably with the ingestion of iodide which is in especially high concentration in some medicaments, e.g. cough mixtures. These are prescribed in fibrocystic disease because of the frequent occurrence of bronchial infections.

CHAPTER 25

AUTORADIOGRAPHY OF THE SALIVARY GLAND

The autoradiographic studies reported by Cohen et al (1955) and Logothetopoulos and Myant (1956a) were performed using a freeze drying technique in order to avoid diffusion of the iodide ion during the preparation of the autoradiographs. Several new techniques have been reported recently to overcome the problems of autoradiography with diffusible substances which avoid the tissue shrinkage associated with the freeze drying method. The experiment of Cohen et al with the hamster submaxillary gland was repeated using the method described in Chapter 7 which is based upon the principle of keeping the tissue and tissue fluid within solidly frozen from the time of removal from the intact animal until the exposure to the autoradiographic emulsion is complete.

METHOD:

500 μc ^{131}I were injected intraperitoneally into 6 adult male hamsters weighing 150 - 160 gs. One hour later under ether anaesthesia the submandibular and sublingual glands were dissected out and placed immediately into a glass container surrounded by carbon dioxide snow and methyl alcohol. Serial frozen sections were cut and the autoradiographs

were prepared using the surface tension technique described on page 44, Chapter 7. This technique is demanding and time consuming and therefore only the submandibular and sublingual glands were used. The main problem is maintaining the low temperature -17^{0}C within the cryostat. This was best achieved by cutting one block at a time.

RESULTS:

Two parts of the duct system can be recognised readily on histological examination. The proximal ducts are intra-lobular and are lined by columnar cells with a granular cytoplasm and the nuclei are situated basally. They have a narrow lumen. The more distal ducts have a larger circular lumen lined by flatter epithelial cells with the nuclei centrally positioned (Figure III, 25).

Sections of the submandibular glands showed an intense autoradiographic image within and around the proximal ducts (Figures III, 25, 26 and 27). The surrounding acinar tissue, the distal ducts and the adjacent blood vessels showed an image only slightly above background levels. The sublingual gland showed negligible concentration of ^{131}I in ducts or acini. In addition, however, radioactivity was detected by this method over the lining of a few of the large distal ducts of both submandibular and sublingual glands.

No radioactivity was present in tissue processed routinely through alcohol, water etc. As a further control some sections were allowed to

thaw slightly using the temperature of the finger applied on the undersurface of the glass slide, and then treated in exactly the same way as the others. When developed these showed a diffuse image over the entire section.

One problem using this method is that cutting serial frozen sections 10 μ thick, it is difficult to obtain duplicate sections for visual comparison. The close relationship of the sublingual gland is fortunate in that a built in control is available (Figures III, 25 and 26).

DISCUSSION:

All sections of the submandibular glands showed an intense autoradiographic image over the proximal ducts but an image only slightly above background in the adjacent acini. These appearances can be interpreted in two ways. Firstly iodide may be concentrated solely in the proximal ducts and the image overlying the acini is due simply to some diffusion of iodide. Secondly both proximal ducts and acini may concentrate iodide but the acini to a lesser degree than the ducts. The first interpretation is not improbable since, as described above, when diffusion is allowed to occur during the preparation of the autoradiographs a diffuse image over the entire gland with no particular localisation in any structure was demonstrated. Further experiments at varying time

intervals after the administration of ^{131}I might resolve this problem. However, the technique described here is exacting and time consuming and is unsuitable for a larger experiment of this nature.

These results are very similar to those obtained by Cohen et al (1955) and Logothetopoulos and Myant (1956a) using the freeze drying technique. These workers found the selective concentration of ^{131}I and ^{35}S-thiocyanate over the proximal ducts of the submandibular glands in hamsters and mice. Logothetopoulos and Myant (1956a) also noted some radioactivity over the distal ducts of the submandibular and sublingual glands in the hamster suggesting that in the former this could result from ^{131}I being carried down in the saliva after secretion from the proximal ducts. In the present study only a few of the larger distal ducts of both submandibular and sublingual glands showed radioactivity.

These autoradiographic studies present and previous, strongly support the dynamic radioisotopic studies of Burgen and Seeman (1957) and Burgen et al (1959) in the dog parotid gland, which suggests that the ducts are responsible for iodide transfer (page 110). They would also be consistent with the inverse relationship between salivary iodide concentration and flow rate in man described in Chapter 17. The salivary iodide concentration decreased linearly with increase in flow rate up to about 1.5 ml/min. Thereafter it remained constant and the

suggestion was made, page 118, that this could be caused if the salivary iodide was mainly produced by the acini at high flow rates when the iodide normally transferred in the ducts was highly diluted. However no direct information is yet available on the site of iodide concentration in human salivary glands.

SUMMARY

Autoradiographic studies of ^{131}I in the hamster submandibular and sublingual glands have been described. The areas within and around the proximal ducts of the submandibular gland show a marked concentration of ^{131}I. The surrounding acinar tissue, the distal ducts and the adjacent blood vessels showed an image only slightly above background level. A few of the larger distal ducts showed more intense radioactivity than the remainder.

There is negligible ^{131}I concentration by the ducts or acini of the hamster sublingual gland although a few of the larger distal ducts showed some radioactivity.

These autoradiographic studies provide further evidence of a ductal site of iodide transfer using a new approach to tissue emulsion contact.

CHAPTER 26

THE SALIVARY IODIDE AND ITS VALUE IN CLINICAL PRACTICE

Many aspects of iodine metabolism can be fully understood only if the concentration of the inorganic iodine (PII) is known (Wayne et al 1964). The PII is therefore an important investigation in the diagnosis and treatment of some patients with thyroid disease. It has already been emphasised (Chapter 18) that because iodide is present in such small quantities in the plasma it cannot be measured directly. As it is present in larger quantities in saliva and urine, there it can be measured chemically. It has been suggested by Vought et al (1963) that as there may be a direct relationship between the salivary iodide and the plasma inorganic iodine (PII), the salivary iodide concentration may be used to assess the PII directly. Figure III, 28 shows the relatively poor correlation obtained when the PII is plotted with the salivary iodide concentration in the same euthyroid subjects. The PII in this study of euthyroid subjects was derived from the specific activity of the urine, after a tracer dose of ^{132}I. In practice, therefore, this method of determining the PII directly gives poor results because of the variation in the salivary iodide concentration with flow rate.

However, as has already been described, Chapter 18, the PII can be derived indirectly from the salivary iodide using the specific activity method This method has the great advantage that it is independent of flow rate since

/the salivary stable iodine/^{132}I ratio remains constant at varying salivary flow rates. Comparing the salivary method with the urinary specific activity method, Harden et al (1965a) have shown an excellent correlation between the PII results obtained by both methods in euthyroid subjects (Figure III, 29).

Most workers have measured the PII from the specific activity of the urinary iodine (Wayne et al 1964). In certain circumstances, however, this method is unsatisfactory. Firstly, in disorders of bladder emptying due to advanced age, emotional factors, or disease states such as prolapse, residual urine may occur and accurately timed collections of urine may be impossible. Secondly, although the urinary iodine is usually inorganic (Riggs 1952, Pitt-Rivers and Tata 1959), in thyrotoxicosis it is known that the urine can contain significant amounts of organic iodinated compounds, (Rall 1950; Berger and Peyrin 1957; Alexander et al 1966), not derived from the plasma inorganic iodine. In contrast, in the saliva of the same patients significant amounts of organic iodine compounds are absent (Chapter 21). Similarly in a patient studied recently with a type of dyshormonogenesis (dehalogenase deficiency) a large amount of labelled organic iodine compounds was present in the urine but only inorganic iodine was found in the saliva (Papadopoulos et al 1966). Therefore, in thyrotoxicosis and in at least one form of dyshormonogenesis falsely high PII values will be obtained using the urinary method because of the error contained in the urinary iodine concentrations. Figure III, 30 shows a

comparison of the false and true values obtained respectively with the urinary and salivary methods. In these situations therefore, the salivary method has advantages.

One disadvantage commonly attributed to the salivary method is the technical difficulties related to saliva collection. Initially, in the present study separated parotid and submandibular saliva was collected but latterly mixed saliva has been used and similar results were obtained. Mixed saliva is easily obtained from practically every patient and the only failures which have been encountered so far in our experience have been 4 patients with advanced Sjögren's Syndrome with gross atrophic salivary gland changes and absent secretion of saliva.

The use of the saliva/plasma ^{132}I ratio as a test to differentiate between athyreotic cretinism and a type of dyshormonogenesis in which there is absence of the iodide trapping mechanism (Hung 1962) has already been discussed (Chapter 19, page 124).

The concentration of radioiodine by the thyroid has enabled radioisotopic visualisation of this gland. This investigation is now a standard diagnostic test and either a scanning technique or a gamma camera can be used. Similar use of the salivary gland iodide concentrating ability to allow visualisation of the salivary glands has not yet been made. A salivary gland scanning technique has been described in the present work in Chapter 6. Because of the low radiation dosage to the subject, the isotope pertechnetate ^{99m}, which is also concentrated by the salivary glands (Chapter 20), was used in preference

to radioisotopes of iodine. The potential value of this method as a clinical diagnostic procedure in salivary gland disease has been suggested.

SUMMARY

Because of the variation of the salivary iodide concentration with flow rate it cannot be used as an index of thyroid function or in the estimation of the plasma inorganic iodine (PII) directly. The salivary iodide concentration may be of value clinically in the indirect measurement of the PII by the salivary specific activity method. In thyrotoxicosis and at least one form of dyshormonogenesis the salivary iodide remains almost completely in inorganic form in contrast to the urine where organic iodinated compounds have been demonstrated. In these conditions therefore and also where disorders of bladder emptying occur the PII is more accurately derived from the specific activity of the saliva than from the urine.

The saliva/plasma ^{132}I ratio can be used clinically in the investigation of "athyreotic" cretinism.

The salivary gland iodide concentrating ability may be of value clinically in radioisotopic visualisation of the salivary glands.

CHAPTER 27

THE SALIVARY & THYROID GLANDS A COMPARISON OF THE IODIDE CONCENTRATING MECHANISM

Phylogenetically the thyroid gland is derived from the primitive salivary glands of protochordates. Iodide was concentrated by these primitive glands and after organic binding was secreted into the pharynx, (Gorbman 1958). With evolution the thyroid gland has become a ductless gland and therefore a gland of internal secretion. Similarities remain however between the thyroid and salivary glands in man and it is the purpose of this chapter to describe these and the differences which exist.

Iodide Concentrating Mechanisms in Salivary and Thyroid Glands

Iodide is present only in minute amounts in the plasma but the thyroid gland has an extremely efficient iodide concentrating mechanism. The iodide taken up is bound to protein and thyroid hormones are synthesised. Indeed, the uptake of radioiodine by the thyroid and the release of labelled hormone into the blood following the administration of a tracer dose are used extensively as tests of thyroid function.

Likewise, iodide is also concentrated in saliva to many times the plasma level (Elmer 1938, Schiff et al 1947, Honour et al 1952). Until recently however, no quantitative studies have been made in saliva at

physiological iodide levels, because of the very small quantities of iodide present. Using a combination of stable and radioiodine techniques the plasma inorganic iodine (PII) and the salivary iodide (SI) concentrations have been measured. The salivary iodide concentration varies inversely with the rate of secretion up to a flow rate of 1-2 ml/min, thereafter the concentration does not fall further (Chapter 17). The absolute amount of iodide secreted in the saliva is proportional to the plasma inorganic iodine concentration (PII). The salivary iodide clearance varies with flow rate unstimulated. The salivary iodide clearance is of the same order as the renal and thyroid iodide clearance (Chapter 18). The parotid salivary iodide clearance is greater than the submandibular iodide clearance. No relationship has been found between the PII and the parotid and submandibular salivary iodide clearance. It is well recognised that changes in the PII outside the normal range are accompanied by an inverse change in the thyroid iodide clearance. Thus when the PII is abnormally high, as after administration of iodide in pharmacological doses, the thyroid iodide clearance falls to low values. Even within the normal range relatively high PII levels are associated with a low thyroid clearance (Wayne et al 1964). The adjustment of the thyroid clearance to PII is not complete, however, and in persons with a high-normal PII, more iodide is accumulated in thyroid than in persons with a lower PII. Thus, in the thyroid, there is an incomplete homeostatic

mechanism tending to maintain the absolute amount of iodide trapped by the thyroid constant. In contrast, there is no such mechanism tending to maintain the salivary iodide secretion constant. In this respect the iodide concentrating mechanism in the salivary and thyroid gland appears to differ.

The saliva/plasma ^{132}I ratio varies with salivary flow rate. At low flow rates the ratio may be 100 or over whereas at high flow rates ratios below 10 are not uncommon, in normal subjects.

In contrast to the thyroid where iodide taken up is organically bound, the balance of evidence suggests that the salivary iodide in man is almost completely in the inorganic form (Chapter 21). Cohen (1962) using trichloracetic acid precipitation claimed to demonstrate organic iodine in human saliva. Other workers have found as in the present studies, mainly inorganic iodide (Freinkel & Ingbar 1953; Ruegamer et al 1955; Gerbaulet and Maurer 1958; Cohen and Myant 1959; Weiss et al 1962). Using a combination of ion exchange resins and radiochromatography no organic compounds were found in the saliva of thyrotoxic patients although some of the iodine was precipitated by trichloracetic acid. These findings have also been confirmed in normal subjects. Organic binding of iodine in

the salivary glands, however, like the iodide concentrating mechanism itself, appears to be subject to species variation and Weiss et al (1962) have shown that a substantial amount of iodine is organically bound in the saliva of dogs.

Various anions, e.g. perchlorate, thiocyanate and nitrate block the trapping of iodide in the thyroid (Wyngaarden et al 1953) and if administered in sufficient dosage hypothyroidism may result. Perchlorate, thiocyanate and nitrate have all been shown to block the salivary iodide concentrating mechanism (Rowlands et al 1953; Edwards et al 1954; Burgen and Seeman 1957; Ferguson et al 1957; Wolff 1964; Mason et al 1966b). A common transport system which is subject to competitive inhibition is the probable underlying mechanism (Edwards et al 1954).

Carbimazole also prevents thyroid hormone synthesis by blocking the organic binding of iodine after trapping has occurred. It does not interfere however, with the iodide trap in the thyroid gland or in the salivary gland (Chapter 22).

The iodide uptake by the thyroid gland is under the control of thyroid stimulating hormone (TSH) secreted by the anterior pituitary gland. With increased secretion of TSH the absolute amount of iodide taken up by the thyroid is increased and vice versa. No similar effect is observed on the salivary glands as determined by

the concentration of iodide in the saliva (Table III, 16). This confirms previous work in man (Myant 1960), in dogs (Ruegamer et al 1955) and in mice (Taurog et al 1959).

Technetium, like iodine a member of the VIIth periodic group, has some similar biological properties to iodine. Both are concentrated by the thyroid gland (Andros et al 1965). Technetium ^{99m} is concentrated in saliva but to a lesser extent than iodide. The concentration of both in saliva falls with increasing rate of salivary flow (Chapter 20). Using $^{99m}TcO_4$ as a clinical tracer the salivary glands as well as the thyroid may be visualised by radioisotopic scanning (Chapter 6) and permits uptake measurements to be made and compared.

DISEASES AFFECTING THE SALIVARY AND/OR THYROID GLAND

Various diseases affect both the salivary and thyroid glands. These will be considered under two headings:-

a) Diseases primarily affecting the thyroid gland, and

b) Diseases primarily affecting the salivary gland.

A) Diseases primarily affecting the Thyroid Gland:

(i) Inborn errors of thyroid hormone synthesis (Dyshormonogenesis):

The thyroid gland may fail to produce a normal amount of thyroid hormone even though it is presented with an adequate supply of iodine. This may be due to an inborn defect in thyroid hormone synthesis.

Absence of the iodide trap both in the thyroid and in the salivary glands has been described (Stanbury & Chapman 1960, and Wolff et al 1964). The saliva/plasma ^{131} ratio may therefore be used in the evaluation of children with athyreotic cretinism to determine whether or not some of these children have congenital goitrous cretinism with absence of the trapping mechanism and have been erroneously called athyreotic. The salivary iodide trap in one patient with a second type of dyshormonogenesis - dehalogenase deficiency, has been studied. This patient excreted large quantities of organic iodine in the urine, but in contrast no organic compounds were found in saliva (Papadopoulos et al 1966).

(ii) Postradioiodine damage

Radioiodine has been used extensively in the treatment of thyrotoxicosis and thyroid carcinoma and in a large number hypothyroidism results (Beling & Einhorn 1961, Dunn & Chapman 1964 and McGirr et al 1964). Occasionally following a large dose of radioiodine a transient parotitis has been observed and Schneyer (1953) has reported a reduction in salivary amylase activity. This reached a maximum between the fourth and tenth day, followed by a gradual return to preirradiation levels. However, in patients who had repeated doses of radioiodine the amylase activity was reduced for much longer periods and in some cases, remained low indefinitely. The salivary flow rate

was also reduced and rampant enamel dental caries of an atypical distribution was reported - the first affected areas being the buccal cervical regions on the premolar teeth (Schneyer & Tanchester 1954).

(iii) Thyrotoxicosis and myxoedema

In thyrotoxicosis or hyperthyroidism the iodide trap in the thyroid gland is overactive and increased amounts of iodine are taken up by the thyroid gland (Wayne et al 1964). The opposite is true in hypothyroidism or myxoedema. The iodide concentrating mechanism of parotid and submandibular salivary glands in euthyroid, thyrotoxic and hypothyroid patients has been studied quantitatively (Chapter 23). The salivary iodide trap was found to be independent of thyroid function. Difference in the salivary flow rates and in the levels of inorganic iodide of the plasma accounted for slight differences which were found in the salivary iodide concentrations in these groups.

In animals, however, there is evidence of both histological change and variation in salivary flow rate in altered states of thyroid function. This work has been reviewed by Shafer and Muhler (1960).

B) Diseases primarily affecting the Salivary Gland

(i) Sjögren's Syndrome:

This disease, described originally by Sjögren in 1933, has aroused considerable interest recently because of a possible auto-immune etiology (Sjögren 1961; Bunim 1961, Anderson et al 1961). The syndrome consists of the triad dryness of the mouth (xerostomia) with or without salivary gland enlargement, dryness of the eyes (kerato-conjunctivitis sicca) and a connective tissue disease usually rheumatoid arthritis (Bloch et al 1965). The xerostomia and kerato-conjunctivitis sicca are the result of reduced secretion by the salivary and lacrimal glands which are the seat of chronic inflammatory change. The histopathological changes in the salivary and lacrimal glands are loss of secretory parenchymal cells with infiltration of lymphocytes and plasma cells; and eventual fibrosis; the normal lobular pattern of the glands is, however, maintained. It is of interest that similar histopathological changes occur in the thyroid glands of patients with Hashimoto's thyroiditis also a disease characterised by the presence in the serum of persistent auto-antibodies (Heaton 1959). Bloch et al (1965) have shown that twenty of sixty-two patients with Sjögren's syndrome had positive tanned red cell tests for thyroglobulin antibodies and of these, five had diffusely enlarged or nodular thyroids. Two of three

who had thyroid surgery were shown to have Hashimoto's thyroiditis and in the third there were foci of interstitial lymphocytic infiltrations.

In a study of patients with Sjögren's syndrome it has been demonstrated (Chapter 24) that the saliva/ plasma ^{132}I ratio may be decreased in this condition as well as the salivary flow rate. In Hashimoto's disease the iodide trap may be normal although the ratio of hormone produced to iodine taken up by the thyroid is abnormally low (Buchanan et al 1961).

(ii) Fibrocystic Disease

The exocrine glands, in particular the pancreas, salivary, sweat and bronchial glands, are affected in this condition. In the parotid saliva for example, the concentrations of sodium, calcium and phosphorus are increased (Chauncey et al 1962) and Bessman (1956) has reported high iodide values. High iodide levels, however, may result from a high plasma iodide concentration due to iodide ingestion in the form of iodide containing cough mixture and tonics (Chapter 24). Neither is there evidence for a defect of iodide handling in the thyroid gland in this condition.

(iii) Post External Irradiation

Xerostomia is a common complaint in patients receiving radiotherapy when the salivary glands lie in the field of treatment. Frank et al (1965) has described progressive involution of the serous cells followed by mucous cells resulting in glandular atrophy and intra-lobular fibrosis. Although the salivary flow rate is decreased the iodide concentrating mechanism appears

to be unaffected (Awwad 1959). Markson and Flatman (1965) have described the development of hypothyroidism in patients treated by external irradiation of the neck region for malignant disease not of the thyroid. This may not, however, be a direct result of irradiation damage but could perhaps be due to the development of thyroid antibodies.

SUMMARY:

The salivary and thyroid glands have been compared in health and disease with particular reference to their iodide concentrating mechanisms. The principal similarities and differences between the two glands are summarised in Table III, 17.

PART IV

STUDIES IN SJÖGREN'S SYNDROME

PART IV

INTRODUCTION

This part of the present work reports the results of studies in 30 patients with Sjögren's syndrome. Sjögren's syndrome consists basically of the triad of xerostomia (dry mouth), keratoconjunctivitis sicca (dry eyes) and rheumatoid arthritis or other connective tissue disease. Recently there has been considerable interest in this condition because of a possible auto-immune aetiology. Some of the techniques for the investigation of salivary gland function, described in Part I, were used to assess the salivary gland involvement in these studies. After a historical review, diagnostic criteria are defined and the general clinical and laboratory findings of each patient described. The oral and salivary gland involvement is assessed using hydrostatic sialography and measurement of salivary flow rate. The results of these two methods are compared and related to the oral symptoms and signs. Labial gland biopsy is evaluated in a controlled series of 8 patients with Sjögren's syndrome and also in 8 patients with 'definite' rheumatoid arthritis. Finally, the incidence of Sjögren's syndrome in patients with auto-immune thyroid disease is investigated.

CHAPTER 28

SJÖGREN'S SYNDROME - HISTORICAL REVIEW

The following are excerpts from the history of a clinical case presented on March 9th, 1888, to the Clinical Society of London by Dr. W.B. Hadden.

> "The patient was a widow, age 65, who came under my care at St. Thomas's Hospital on December 1, 1887..... .... She stated that about seven months before she came under my notice her mouth gradually began to get dry, and that the dryness had steadily increased. To relieve the discomfort caused by the want of natural moisture she had to be constantly sipping fluid. She complained, too, that the act of swallowing was difficult and often painful ..... Nearly all her teeth were wanting, having been extracted nearly twenty years previously. The tongue was red, devoid of epithelium, cracked in all directions like crocodile's skin, and absolutely dry. The inside of the cheeks, the hard and soft palate, were also quite dry; the mucous membrane was smooth, shiny, and pale, but here and there a few patches of injection were seen. The tonsils were of normal size and appearance. The back of the pharynx was natural in colour, but there was a marked deficiency of moisture.... .... I may mention here that during her stay in the hospital and whilst she was improving, the woman usually noticed some moisture in the mouth when she began to masticate ..... The woman told me that about two months before admission she had occasion to cry, but no tears would come. The conjunctivae appeared to be natural, but on getting her to smell a strong solution of ammonia the lachrymal secretion was not stimulated in the least, although the same solution brought the tears welling to my eyes at once ....."

The above is probably the first recorded description of Sjögren's syndrome. It is an excellent account of the combined oral and ocular dryness, i.e. the 'sicca syndrome.' Although several earlier reports including Hadden's had appeared (Hadden 1888, Fuchs 1919, Gougerot 1926, Betsch 1928, Isakowitz 1928, Chamberlin 1930) it was not until 1933 that Henrik Sjögren, a Swedish Ophthalmologist from Jonköping, fully documented the association of lacrimal and salivary gland involvement in the syndrome which bears his name. In his initial series, Sjögren (1933) described 19 female patients mostly postmenopausal of whom 13 had arthritis and 6 the 'sicca syndrome,' i.e. the combined oral and ocular dryness. In addition, he also described rhino-pharyngolaryngitis sicca and enlargement of parotid and less frequently, the submandibular glands.

It later became apparent that rheumatoid arthritis was the joint disease related to this syndrome (Stenstam 1947) and the frequency of rheumatoid arthritis in various reported series of Sjögren's syndrome has varied from 17% to 87% (Bloch et al 1965). Conversely, kerato-conjunctivitis sicca, i.e. "dry eyes" has been reported as occurring in 9% to 34% of patients with rheumatoid arthritis (Bunim 1961). The components of the sicca

complex have been described in association with systemic lupus erythematosus (Bain 1960; Morgan 1954; Ramage and Kinnear 1956; Shearn 1961), polyarteritis nodosa (Ramage and Kinnear 1956), progressive systemic sclerosis (Ramage and Kinnear 1956; Shearn 1960; Stava 1958; and Bloch et al 1965), and polymyositis (Silberberg and Drachman 1962).

A clinical syndrome involving the salivary and lacrimal glands has been given the name of Mikulicz's syndrome or disease. Mikulicz (1892 translated 1937) described a 42 year old male patient (Figure IV, I) with bilateral symmetrical enlargement of the parotid, lacrimal, submaxillary, buccal and palatal glands but there was no description of dryness of the eye or mouth. Biopsy of the lacrimal and submaxillary glands revealed considerable round cell infiltration and atrophyof the secretory parenchyma. Mikulicz interpreted the findings as indicating a low-grade infection. The nature of Mikulicz's Disease and its relationship to Sjogren's syndrome were aspects which caused confusion and Thursfield (1914) attempted to clarify the position by classifying cases of Mikulicz's disease on an aetiological basis. In 1927 Schaffer and Jacobsen modified Thursfield's classification and defined two main groups of salivary gland disease bearing Mikulicz's name.

(1) Mikulicz's disease of unknown etiology and having a benign course.

(2) Mikulicz's syndrome which consisted of a number of specific diseases and disorders affecting the salivary and/or lacrimal glands, e.g. tuberculosis, sarcoidosis, leukaemia, lymphosarcoma, lead and iodide poisoning.

Morgan and Castleman (1953) and Morgan (1954) on the basis of their clinical and histopathological studies have suggested that group (1), i.e. Mikulicz's disease may be a variant of the larger symptom complex of Sjögren's syndrome.

> Morgan (1954) states - "A re-examination of the clinical records of the 18 patients previously considered to have Mikulicz's disease disclosed that a significant number had other components of Sjögren's syndrome such as kerato-conjunctivitis sicca, xerostomia, and rheumatoid arthritis.
>
> Conversely, a study of cases classified as Sjögren's syndrome revealed that the incidence of the several components of Sjögren's syndrome was extremly variable, and in addition, many of the patients had chronic enlargement of the salivary glands, one of the prominent features of Mikulicz's disease.
>
> The condition characterised by chronic enlargement of salivary and lacrimal glands which in the past has been called Mikulicz's disease, may be a less highly developed variant of a larger symptom complex Sjögren's syndrome.
>
> Because of the high incidence of rheumatoid arthritis in Sjögren's syndrome it is possible that the pathologic process in the salivary or lacrimal glands is similar and of systemic origin."

This concept that Mikulicz's disease and Sjögren's syndrome were a single entity has now become accepted (Lennox 1960; Bunim 1961; Waterhouse 1963).

In 1958 Jones described precipitating antibodies reacting with extracts of salivary and lacrimal glands in a few patients with Sjögren's syndrome. He suggested that the salivary and lacrimal glands involved in Sjögren's syndrome may have common antigens and that an antibody formed after damage to a gland could cause generalised salivary and lacrimal gland changes. It is noteworthy, however, that the antibodies Jones detected in these patients also reacted with extracts of human kidney, and were therefore non-organ specific. In a much more detailed study Anderson et al (1961) found precipitating auto-antibodies reacting with extracts of human and animal tissues in patients with Sjögren's syndrome. The antibodies were non-organ specific and were distinct from auto-antibody to thyroglobulin. In 1962 Heaton reported antithyroid auto-antibodies present in 52%, antinuclear factor in 48%, the auto-immune complement fixation test for salivary and lacrimal glands, liver and kidney, 49% and rheumatoid factor 82% in a clinical and serological study of patients with Sjögren's syndrome.

An important basic contribution to our understanding of Sjögren's syndrome has come from the work of Bunim and his colleagues (Bunim 1961; Bloch and Bunim 1963; Bloch et al 1965). They have shown in a combined clinical and serological study of 62 patients the inter-relationship of rheumatoid arthritis and other connective tissue diseases, such as progressive systemic sclerosis, polymyositis, systemic lupus erythematosus, in association with components of the sicca complex. They have demonstrated that the incidence of rheumatoid factor in patients with the sicca complex alone is similar to patients with 'definite' rheumatoid arthritis. Other typical serological reactions found were the occurrence of anti-nuclear factor (77%), complement fixing antibody to liver homogenate (49%), and thyroglobulin antibodies (27%); findings which were similar to those reported by Heaton (1962).

From the results to date, therefore, it is apparent that Sjögren's syndrome has serological aspects in common with systemic lupus erythematosus and auto-immune thyroiditis. Clinically and serologically there is good evidence for placing it within the group of diseases in which there is a disturbance of the immune mechanisms, and which have been designated the auto-immune diseases (Mackay and Burnet 1963).

CHAPTER 29

DEFINITION, PATIENTS STUDIED, CLINICAL AND LABORATORY FINDINGS

1) DEFINITION

2) PATIENTS STUDIED

3) CLINICAL FINDINGS - GENERAL

4) LABORATORY FINDINGS

DEFINITION:

Sjögren's syndrome consists of the triad of xerostomia (dry mouth), keratoconjunctivitis sicca (dry eyes), and rheumatoid arthritis. From recent work (Bunim 1961, Bloch et al 1965), it is apparent that another connective tissue disease may be present instead of rheumatoid arthritis as outlined in Chapter 28.

Salivary gland or lacrimal gland enlargement may or may not be present. The term 'sicca syndrome' refers to the association of kerato-conjunctivitis sicca and xerostomia. Two of the three major components are generally considered sufficient for making a diagnosis of Sjögren's syndrome but excluded from the diagnosis are specific disease of the salivary or lacrimal glands, e.g. sarcoidosis, lymphoma and tuberculosis.

Histologically the syndrome is characterised by replacement of the acinar parenchyma by lymphoid tissue and an intra-ductal proliferation of two cell elements, epithelial and myoepithelial with the formation of epimyoepithelial islands, (Morgan 1954) Figure IV, 2.

PATIENTS STUDIED:

All the 30 patients included in this study had at least two of the three major components of Sjögren's syndrome. Twelve of the patients had 'definite' rheumatoid arthritis diagnosed on the basis of the criteria of the American Rheumatism Association (Ropes et al 1958).

Every patient complained of xerostomia either on presentation or after questioning. Eight patients presented with xerostomia, 10 with kerato-conjunctivitis sicca, 7 with rheumatoid arthritis and in the remaining 5 patients, angular cheilitis, neck swelling, angina, weight loss and osteoarthritis were the presenting complaints.

The 30 patients were divided into two groups, A. and B. Group A consisted of 12 patients who had rheumatoid arthritis and sicca components and Group B the 18 patients who had the 'sicca syndrome' alone. The duration of the three major

components are shown for each patient in Table IV, 1.

CLINICAL FINDINGS (Table IV, 2)

(a) Age and Sex Distributions. Twenty-six females and 4 males were included in the patients studied. The total age range was 47-78 years with a mean of 61.8 years. The age range and mean age in the two sub-groups were, Group 'A' - range 47-78 years, mean 61.0 years, and Group 'B' range 53-73 years, mean 62.3 years.

Age of onset of first Sicca Component. The age range of onset of the first sicca component for all patients was 31-76 years and the mean age 51.8 years. Comparable figures for the two sub-groups were:-

Group 'A' - Age range 31-76 years, mean 51.0 years.

Group 'B' - Age range 48-70 years, mean 52.4 years.

At the time of examination the duration of sicca components was on average 9.8 years in 'Group A' and 4.7 in 'Group B'. In 20 of the 30 patients studied, kerato-conjunctivitis sicca and xerostomia developed, apparently together, within a period of one year. In 7 patients xerostomia started before kerato-conjunctivitis sicca and in 3 patients, kerato-conjunctivitis sicca

presented before xerostomia (Table IV, 1).

Relation to the Menopause. The relationship of the onset of the first sicca component to the menopause was studied. In 6 patients the first sicca symptom started at the time of, or within a 2 year period of, the menopause. Three patients developed a sicca component before the menopause and in 15 patients sicca symptoms began 3-21 years after the menopause. In 2 patients the approximat time could not be determined because of surgical treatment. It is concluded that the onset of Sjögren's syndrome had no well defined relationship with the menopause.

OPHTHALMOLOGICAL EXAMINATIONS:

All patients examined with one exception (A9) had evidence of kerato-conjunctivitis sicca. This was confirmed by the five minute Schirmer test for tear secretion, rose-bengal staining of the conjunctiva and the finding of filamentary or punctate keratitis on slit lamp examination (Bloch et al 1965).

JOINT INVOLVEMENT:

Twelve patients had 'definite' rheumatoid arthritis. The arthritis was no different from that seen in patients who did not have Sjögren's syndrome and several patients had knee joint

effusions aspirated i.e. they did not have 'dry joints'. There was also no obvious difference in the incidence of subcutaneous nodules and other visceral involvement in this small series of patients as compared to rheumatoid patients without Sjögren's syndrome.

OTHER RESULTS:

Only 4 patients had actual salivary gland enlargement by history and/or on examination. Seven patients had co-existent thyroid disease, 3 had Raynaud's phenomenon, 3 had anaemia (2 iron deficiency and 1 pernicious anaemia) and 1 had myasthenia gravis. The association of Sjögren's syndrome and thyroid disease will be dealt with more fully in Chapter 32.

LABORATORY INVESTIGATIONS:

Methods: The serum globulins were measured using the salt fractionation method. White blood count, E.S.R., and haemoglobin were estimated by standard laboratory procedures. The following serological tests were performed, (1) tanned red cell haemagglutination test (T.T.R.C.) for thyroglobulin auto-antibodies (Fulthorpe et al 1961). Starting dilutions at 1:16 were employed. (2) Hyland Latex agglutination (R.A.) test and sensitised sheep cell agglutination (S.S.C.A.) test (Ziff et al 1956).

Starting dilution for S.S.C.A. was 1:32. (3) precipitin tests for antibodies to cellular constituents (Anderson et al 1962b), using undiluted serum. (4) L.E. cell test and Hyland Latex agglutination (L.E.) test for anti-nucleoprotein. (5) fluorescent antibody studies for antinuclear factor (A.N.F.), (Beck 1961). The lowest serum dilution tested for A.N.F. was 1:16. The 1:16 starting dilutions were prepared for A.N.F. and T.T.R.C. tests in view of the high frequency of positive tests at lower dilutions in hospital populations (Beck 1961, Hackett et al 1960). When positive results were obtained with the screening tests the sera were titrated at fourfold dilutions. The morphological patterns of nuclear staining, e.g. "homogeneous" "speckled" and "nucleolar" produced by antinuclear auto-antibodies were defined by the criteria described by Beck (1961, 1962, 1963).

RESULTS:

The results of these laboratory investigations are detailed in Table IV, 3.

Three of the 30 patients (A11, B3 and B12) had haemoglobin values of less than 10g/100 ml. On more detailed examination of these patients 2 had simple iron deficiency anaemia and 1 had pernicious anaemia as defined by achlorhydria with augmented

histamine test, megaloblastic bone marrow and abnormal Schilling test corrected by intrinsic factor. This patient also had antibody to gastric parietal cells as detected by an indirect immunofluorescence technique (Adams et al 1964). Five patients had a total white cell count less than 4,000/cmm and one of these had a count less than 3,000/cmm (patient B12 who also had pernicious anaemia). Differential white cell counts in these patients with leucopenia showed a relative lymphocytosis. The erythrocyte sedimentation rate was elevated (above 15 mm in the 1st hour) in 22 patients, especially so in those with rheumatoid arthritis. The total serum globulins exceeded 3g/100 ml in 19 patients, the highest serum globulin being 4.5 g/100 ml in a patient (A7) with rheumatoid arthritis.

An interesting finding was the presence of rheumatoid factor as determined by the Hyland R.A. test in all but 6 patients studied. These 6 patients all had the 'sicca syndrome' alone. The sensitised sheep cell agglutination test (SSCA) was less frequently positive than the Hyland R.A. test especially in those patients with the 'sicca syndrome' alone. Rheumatoid factor has been shown by an immunofluorescence technique to be produced

by the plasma cells surrounding subcutaneous nodules and germinal centre cells of lymph nodes adjacent to the inflamed joints (Mellors et al 1961). Where it arises from in patients who only have the 'sicca syndrome' is not known but it is of interest that Bunim et al (1964) have reported one patient in whom rheumatoid factor was present in the plasma cells within the parotid gland and cervical lymph nodes. A high incidence of rheumatoid factor in patients with the 'sicca syndrome' only has been reported previously by Bloch et al (1965).

The L.E. latex and L.E. cell tests were negative in all patients. Heaton (1959) found a high incidence of L.E. cell phenomena in Sjögren's patients studied but the clinical findings of many of the patients are not fully detailed and it is uncertain how many had rheumatoid arthritis. In the study of Bloch et al (1965) L.E. cell tests were only positive in 8% of the patients examined and all of these had rheumatoid arthritis. However, patients with Sjögren's syndrome and systemic lupus erythematosus have been reported in the literature although none were found here. The more sensitive test for antibody to cell nuclei, i.e. antinuclear factor test, was positive in 16 of the 30 patients studied and reached a titre of 1 in 1000 in 3 patients. The antinuclear factor test was more frequently positive in higher

titres in those patients with Sjögren's syndrome who had rheumatoid arthritis than in those patients who had sicca components alone. This finding is contrary to that of Bloch et al (1965) who found that the incidence and titre of antinuclear factor was higher in those with the 'sicca complex' alone. The discrepancy between these results may be accounted for by the less clinically severe cases with the 'sicca syndrome' alone in the present study. In the study of Bloch et al the patients were obtained by clinical selection from hospitals throughout the U.S.A. on the basis of their clinical severity (Buchanan 1966). Precipitating antibodies to tissue extracts (distinct from anti-thyroglobulin) were present in 8 patients 3 of whom had the 'sicca syndrome' alone. Six patients had anti-thyroglobulin autoantibodies (20%) and of these 5 were of relatively high titre (1:4000). The significance of this finding will be discussed in more detail in Chapter 32.

It is clear from the above clinical and serological findings and also from those of Bloch et al (1965) that Sjögren's syndrome is associated with a wide spectrum of autoimmunity both of non-organ and organ specific type. Antibodies to the cytoplasm of salivary gland ducts have been reported by Bertram and Halberg (1964) in Sjögren's syndrome but these apparently cross react with a number of other tissues, e.g. breast (Anderson et al 1966

unpublished). Further work with this auto-antibody is clearly required and may help to elucidate the enigma of the immunological changes found in this disease. Sjögren's syndrome challenges many serious diseases such as systemic lupus erythematosus in the number and diversity of the auto-antibodies found. The benign course of Sjögren's syndrome is evidence against the direct pathogenic role of circulating auto-antibodies although it is noteworthy that antibodies to desoxyribonucleic acid have not been found in Sjögren's syndrome. This antibody, whose role is undetermined, is present in a proportion of patients with systemic lupus erythematosus and in particular those whose illness runs a fulminating course (Casals et al 1964).

CHAPTER 30

ORAL AND SALIVARY GLAND INVOLVEMENT

Rheumatoid arthritis and kerato-conjunctivitis sicca are diagnoses readily made by well defined criteria. The position is less clear with regard to xerostomia. Some authors accept the history and appearance of dry mouth as proof of the presence of this sicca component (Allington 1950), while others require sialographic evidence or the results of salivary flow studies (Bloch et al 1965). Clinical studies of more than a few patients have seldom been recorded. In this chapter I shall describe studies of the oral symptoms and signs and investigations of salivary gland function in the 30 patients whose general clinical and laboratory results have been reported in the preceding chapter.

METHODS

a) History taking: Each patient was questioned about the following symptoms: duration and nature of oral dryness, difficulty in mastication, e.g. food sticking to dentures or oral mucosa, difficulty in swallowing, increased fluid intake (during meals, in general and/or having fluids available at

bedside during the night), abnormalities of taste, oral soreness, ill-fitting dentures, oral ulceration and fissuring or ulceration of lips. The patients were then examined for any oral signs. Dryness of the mouth, fissuring of the tongue, angular cheilitis or cheilosis, ulceration, dental caries, denture trauma etc.

b) Sialography: As already described in Chapter 4 the hydrostatic technique of sialography was developed because of problems relating to the use of this procedure in patients with Sjögren's syndrome. Figure I, 13, shows the results of sialography using a hand injection technique in a patient with a markedly atrophic gland. There is marked extravasation of contrast medium into the surrounding tissues. This is especially liable to occur in atrophic glands as, in this condition and especially when the hand injection technique is employed, the filling pressure cannot be adequately controlled.

The hydrostatic technique was therefore developed using sodium metrizoate (Triosil '45') as the water soluble contrast medium (as described in Chapter 3). The contrast medium entered the duct through a tapered polyethylene catheter from a glass container positioned 70-90 cms above the patient's head. Using this method overfilling of the gland

rarely occurred. A film was taken at the completion of the filling phase. As the water soluble contrast medium is rapidly expelled from the glands and ducts a secretory phase film was taken five minutes after completion of the filling phase. Between the two phases salivary flow was stimulated with lemon juice.

c) Measurement of Salivary Flow Rate In the tests of salivary gland function the salivary flow studies were carried out on the same gland (right or left parotid) which had been examined sialographically. In 23 patients parotid saliva was collected using a modified Carlson-Crittenden cup or by cannulation of the parotid ducts as described by Kerr (1961). The saliva was passed via polyethylene tubing through a drip chamber and each drop recorded using the photo-electric detector system. These methods are described in detail in Chapters 1 and 2.

Saliva was collected under 'resting' conditions and after fruit gums, lemon juice and occasionally paraffin wax stimulation. The collections were continued until 1 ml. of saliva was obtained at each flow rate but this was not always possible especially under 'resting' conditions.

RESULTS

a) Oral Symptoms and Signs associated with Xerostomia

These are recorded for each patient in Tables IV, 4 & IV, 5. All the patients complained of oral dryness, in 18 patients it occurred intermittently, and in 12 patients it was persistent. Difficulties with mastication; increased fluid intake with meals, and in general; fissuring or ulceration of the lips; were all described by over 50% of the patients questioned. A dry mouth was present in 19 of the 30 patients examined. Fissuring of the tongue and lips was also commonly observed (Figures IV, 3 - IV, 5).

Taste abnormalities, oral ulceration and difficulties in swallowing were reported by a few patients only. Any oral ulceration present was diagnosed from the history or found on subsequent examination to be of traumatic or aphthous origin in the few instances it did occur. Three patients complained of a "brown skin" peeling off the lips and mouth each morning, a symptom previously reported by Bloch et al (1965) in their series. In 3 patients a previous diagnosis of "allergy" to plastic dentures had been made elsewhere and one patient had been advised she was allergic to a plastic

artificial eye before kerato-conjunctivitis sicca was diagnosed. The former were not in fact true "denture allergies" but due to trauma from artificial dentures which were generally less well tolerated in the most severely affected patients. The most common denture complaint, not surprisingly, in view of the decreased amount of saliva was looseness and this would of course contribute to chronic trauma of the oral mucosa. Another patient was diagnosed erroneously as having a mixed parotid tumour 10 years previously.

Only three patients had natural teeth present, and in two of these the incidence of dental caries had been noted to increase recently since xerostomia occurred. It was a disappointing feature of the clinical study that so few patients had natural teeth. In all edentulous patients however the complaint of xerostomia arose before the insertion of artificial teeth and in none of these was a history of rampant dental caries described.

The oral symptoms and signs of groups 'A' and 'B' were compared but no appreciable differences were found (Table IV, 6). However, oral symptoms and signs associated with xerostomia were considerably more common in patients

with abnormal sialograms than in those with normal sialograms (Table IV, 7).

b) Sialography: Of the 30 patients studied 14 had normal sialograms and 16 had abnormal sialograms (Table IV, 8). The 16 abnormal sialograms were classified on a purely morphological basis as punctate, globular, or cavitary, as described by Rubin and Holt (1957), and atrophic, where marked diminished arborisation of the duct system was apparent. Typical examples are shown in Figures IV, 6 - IV, 11. Punctate sialectasis was present in 6, globular in 4, and cavitary in 3 patients. Atrophic changes were present alone in 3 patients and accompanied sialectasis in 6 patients. A comparison between Group 'A' and Group 'B' show 58% and 50% had abnormal sialograms respectively. No difference in the type of abnormalities was apparent between the two groups.

c) Salivary Flow Rate: Of the 23 patients examined 12 had abnormal and 11 normal sialograms. Under 'resting' conditions no saliva was collected from 9 patients (Table IV, 9). In all of these patients with one exception (B18) sialograms were abnormal. In 1 patient the salivary flow rate under 'resting' conditions

was diminished and in another 3 patients with abnormal sialograms the 'resting' flow rate was within the author's normal range (Figure IV, 12).

After stimulation with fruit gums and lemon juice the salivary flow rate was abnormally low in 10 patients and all of these had abnormal sialograms. In 4 of these patients no saliva was collected even after maximal stimulation, two of these patients (A4, B4) had cavitary sialectasis, another (B17) globular, and the fourth (A1) extreme atrophic changes. Ten of the 11 patients with normal sialograms had normal salivary flow rates. A typical tracing obtained on the photo-electric drop recorder in a patient (A2) in whom the salivary flow was diminished but not absent is shown in Figure IV, 13.

On comparing the salivary flow rates of Group 'A' and Group 'B' patients when high flow rates are studied, a decreased flow is apparent in Group 'A' and Group 'B', in comparison with a control series suitably matched for age and sex (Table IV, 9). When the salivary flow rates of 10 patients with abnormal sialograms are compared with the flow rates of 10 patients with normal

sialograms a significant reduction in salivary flow rate is observed (Table IV, 10) - at both low and high flow rates $P < 0.005$. All of the patients examined with normal sialograms have in fact salivary flow rates within the author's normal range (Figure IV, 12), and similar to the control group. In Figure IV, 12, the salivary flow rates in Sjögren's patients with abnormal sialograms are seen in relation to the values obtained in 93 normal subjects.

DISCUSSION

Oral symptomology is a common manifestation of Sjögren's syndrome. The diagnosis may be made late because the initial symptoms may be vague and confined to the oral cavity (Calman and Reifman 1966 and Jacobson 1966). All the patients in the present series complained of xerostomia. This dryness resulted, in the majority of patients, in difficulties with mastication due to food sticking to dentures and to the oral mucosa, increased fluid intake, and fissuring of tongue and lips. About 30% experienced oral soreness or discomfort in the absence of an obvious cause. Loose upper and lower artificial teeth was also quite a common complaint. Other workers have reported an increased incidence of

dental caries associated with Sjögren's syndrome but as 27 of the 30 patients in the present series were edentulous before the development of xerostomia this aspect could not be studied.

Xerostomia may be caused by such varying conditions as congenital hypoplasia of the salivary glands to temporary dryness due to mouth breathing, excessive speaking and normal emotional reactions (Allington 1950). Kitamura and Okuda (1962) have shown that the decrease in salivary flow rate is more marked when the dryness of the mouth is associated with salivary gland disease than when of psychoneurotic origin. In the present study 11 of the 23 patients examined, all of whom had complained of xerostomia, had decreased salivary flow rates in comparison with the author's normal range. Ten of these patients had abnormal sialograms. Another two patients with abnormal sialograms had low salivary flow rates but within the normal range. Of the remaining 11 patients with normal sialograms 10 had salivary flow rates within the normal range. Two of the three patients from whom no saliva could be collected had cavitary sialectasis, and the third had extreme atrophic changes. As suggested by Thackray (1955) and Bloch et al (1965) the cavitary appearance may be due to rupture of a

weakened wall in an atrophic gland as a result of pressure during the sialographic filling phase. Using a hand injection technique this is more likely to occur (Figure IV, 14). In one previous survey (Bloch et al 1965), using a hand injection technique, 7 out of 37 patients were found to have cavitary or destructive sialectasis and a fairly good correlation between the severity of the sialectasis and the degree of xerostomia was found.

The present study shows a good correlation between salivary flow rate measurements and the appearances on sialography. Both sialographic appearances and salivary flow rate measurements show a good correlation with the oral symptoms and signs recorded. However, 10 of the 30 patients investigated had neither sialographic abnormalities nor decrease in salivary flow rate to confirm their sensation of xerostomia. It is clear that the subjective sensation of dry mouth and the appearance clinically of xerostomia are not always associated with decreased salivary gland function. This is to be expected as dryness of the mouth is not an uncommon complaint in apparently normal subjects. The clinical appearance of dry mouth, unless of severe degree,

is also an unreliable index of decreased salivary gland function. If Sjögren's syndrome is suspected it would appear from the studies reported that either sialography or salivary flow rate measurements are desirable to confirm the clinical suspicion. Both tests are easily carried out using the techniques described and a good correlation is shown when the results are compared.

Ten (33%) of the 30 patients described above remain who showed no abnormalities using these tests of salivary gland function. Several possible explanations occur:

a) That the total salivary gland function was decreased and the single gland investigated in these patients did not reflect salivary gland function in general.

b) That they have early Sjögren's syndrome.

c) That these patients do not in fact have Sjögren's syndrome.

It is impracticable to measure the salivary flow rate from all glands in one subject at the one visit and several examinations are inconvenient for these patients who are quite often crippled with advanced rheumatoid arthritis. The collection of whole saliva is less accurate and also

maximal stimulation using gustatory stimuli cannot be achieved satisfactorily without rendering the measurement of salivary volume inaccurate. Chemical methods of stimulating salivary gland function with parasympathomimetic drugs as described earlier (page 16) can have unpleasant side effects when given in sufficient dosage to promote high flow rates and are also contra-indicated in certain disease states.

It is quite likely that some of these patients described may have early Sjögren's syndrome. As there is a wide range of 'normal' salivary flow rates (Figure IV, 12) the measurement of salivary flow rate could not be regarded as a sensitive test. The sialographic changes give very similar results to the flow rate measurements and neither of these tests could be regarded as suitable for the early diagnosis of the oral component of Sjögren's syndrome. The question also arises whether Sjögren's syndrome as normally defined and as described on page 184 is a single nosological entity or if it does in fact constitute a heterogeneous group of diseases. It was felt that histological studies might provide further evidence on these points and the results in a small controlled series of patients are reported in the next chapter.

CHAPTER 31

HISTOPATHOLOGICAL FINDINGS

Involvement of the minor salivary glands in Sjögren's syndrome has been recorded previously in single case reports. Different intra-oral sites have been described; the palatal glands (Nelson et al 1963, Cifarelli 1966), the buccal glands (Ellman et al 1951, Bain 1960, and Calman and Reifman 1966) and the small glands of the tongue (Bahskar and Bernier 1960). It is of historical interest that in the patient whom Mikulicz originally described in 1892 the minor oral salivary glands were affected in the disease process.

Biopsy of a major salivary gland (parotid or submandibular) is usually not justified in patients with Sjögren's syndrome unless glandular enlargement is present and a diagnosis of neoplasia cannot be excluded. It may also be difficult to perform as the diseased glands are often markedly atrophic. There is evidence that other mucus secreting glands throughout the body may be affected in this condition (Bucher and Reid 1959, Bloch et al 1965). It would seem reasonable therefore to assess the value of intra-oral biopsy of minor salivary glands as a diagnostic aid in Sjögren's syndrome.

METHOD

The lower lip was chosen as a suitable site for biopsy examination. It has many small mucus secreting glands just below the oral mucosa (Figure I, 43). Their normal appearance is shown in Figure I, 44. This area has an excellent blood supply and is easily accessible.

Three groups of patients were studied

1) 8 patients with Sjögren's syndrome.
2) 8 patients with 'definite' rheumatoid arthritis.
3) 8 control patients with arthritis of non-rheumatoid type.

The three groups were similar for age and exactly matched for sex.

Labial biopsy was carried out as described in Chapter 8, pages 49, 50 and illustrated in Figure I, 42. The injection of local anaesthetic solution (Xylocaine 2%) followed by the removal of tissue took approximately 8-10 minutes. After routine fixation and paraffin embedding separate cuts were made and 3 slides prepared from different areas. All sections were stained with haemotoxylin and eosin.

HISTOPATHOLOGICAL GRADING

On histopathological examination the following abnormalities were observed - accumulation of lymphoid especially around intra-lobular ducts, acinar atrophy, fibrosis, and a few early duct changes. A classification based on the number of foci of lymphocytes in and around the glandular tissue was used. An accumulation of 50 or more lymphocytes was necessary before a focus was diagnosed. Appearances were graded + ≡ 1 focus and ++ ≡ 3 or more foci. Some plasma cells and histiocytes were often demonstrated but the lymphocyte was by far the predominant cell present in these foci (Fig. IV, 15). In the more marked degrees of glandular involvement the foci became confluent. In three patients although actual focus formation did not occur diffuse lymphocytic infiltration was present.

RESULTS

These are summarised in Tables IV, 11, 12, and 13 along with the serological findings. The sialographic appearances and parotid flow rate studies are also included (Table IV, 11) for the Sjögren's patients. In Group (1) 7 (87.5%) of the patients with Sjögren's syndrome had foci of lymphocytes. Of these 4 (50%) had three or more foci and 3 (37.5%) had one

focus. The remaining patient in this group (B1) had diffuse lymphocytic infiltration. In the rheumatoid arthritis patients i.e. Group (2) 4 (50%) of the patients had foci (all had < 3 foci). In the control series of patients with non-rheumatoid polyarthritis i.e. Group (3) none of the patients had foci of lymphocyte accumulation.

These histological findings were those of the author who also performed the biopsies. The sections were later examined without prior knowledge of the clinical diagnosis by Dr. R.B. Goudie, Senior Lecturer in Pathology, Western Infirmary, Glasgow, who graded them according to the extent of round cell infiltration. The only difference between the two assessments was with respect to patient B1 (i.e. the Sjögren's patient without focal lymphocytic adenitis). Dr. Goudie felt because of the diffuse lymphocytic infiltration present this patient had more severe changes than indicated by the author's classification although he agreed that actual foci were not present.

Seven of the 8 patients with Sjögren's syndrome examined had abnormal parotid sialograms and 5 of these had decreased parotid salivary flow rates. Patient A5 had a normal sialogram

and normal salivary flow rates but on histological examination showed a focus of lymphocyte infiltration.

DISCUSSION

Although only a small series of patients has been investigated these results show a definite association between foci of lymphocytes involving the labial mucous glands in both Sjögren's syndrome and rheumatoid arthritis. Evidence that the association is with both of these conditions is provided by the positive results in two patients B2 and B10 who had the 'sicca complex' alone. The degree of involvement was considerably more severe in the Sjögren's group. The typical appearances of Sjögren's syndrome with ductal aberrations and the formation of epi-myoepithelial cell islands were not found in any of the sections examined.

Waterhouse (1963) in a post mortem study of submandibular, parotid and lacrimal glands in a series of 239 subjects found focal lymphocytic adenitis in at least one salivary or lacrimal gland in 70% of females and 30% of males examined. Waterhouse defines focal adenitis of these major salivary glands as 2 or more foci of 50 lymphocytes in the one gland specimen. In view of the

sex incidence he observed it is of interest that in the present series the only Sjögren's subject without focal adenitis was male. This patient however as described above had diffuse lymphocytic infiltration. Waterhouse (1963) believed that focal adenitis of salivary glands is a focal form of Sjögren's syndrome and is akin to focal thyroiditis which has been described by Williams and Doniach (1962) as a "miniature" Hashimoto's thyroiditis. Waterhouse also observed that focal adenitis of the major salivary and lacrimal glands was more common in subjects who had suffered from rheumatoid arthritis.

The lack of ductal changes was a feature of the histopathological findings. Whether this was due to the lack of severity of the cases examined, the smallness of the sample, or reflected a later involvement of these minor glands in the disease process is not known. It might also be a feature of the condition when mucous glands alone are involved.

The normal appearance of these minor salivary glands has not been defined. Cifarelli (1966) in a post mortem series of 15 found no lymphocytic infiltration in 14 and a few lymphocytes were present in the remaining subject. His autopsy

specimens were taken from the palatal region. In view of the present results I have examined a small post mortem series of labial glands. Of 11 autopsies examined so far 10 were unremarkable histologically, 1 (female) showed diffuse lymphocytic infiltration. It was of interest that this subject suffered from rheumatic heart disease a condition in which Waterhouse (1963) found increased incidence of focal adenitis of the major salivary glands. It will be necessary however to examine a large post mortem control series before the normal appearance is completely defined but the evidence at present, from the two small post mortem series cited and the control group examined in the present study, would suggest that focal lymphocytic adenitis does not normally occur in the minor oral salivary glands.

There is evidence that lymphoid tissue within the major salivary glands occurs more frequently in the over 45 year age group (Waterhouse and Doniach 1965), and is more common in females. This would suggest that some factor predisposes to its appearance and its source requires careful consideration. The possibility that they represent an inflammatory process appears unlikely as there was no history of an acute episode

and there was a complete lack of these foci in the control series. In the majority of sections examined there were submucosal lymphocytes present but they were just as prevalent in the control series as in the Sjögren's group. No other histological signs of virus was present and an inflammatory reaction is not a feature of salivary gland virus disease (Rauch 1959, cited by Waterhouse 1963). Neoplastic conditions involving the lymphocyte series are relatively rare and although it cannot be conclusively proven it seems unlikely that the lymphocyte foci seen in the biopsies of the patients studied could be a manifestation of a benign lymphoid neoplasm. It is noteworthy however that lymphoma outwith the salivary and lacrimal glands has been reported as occurring in the course of benign Sjögren's syndrome unassociated with rheumatoid arthritis (Talal and Bunim 1964, Bunim et al 1964, Bloch et al 1965).

The actual role of the lymphocytes in these salivary glands therefore is not known and requires further investigation. Various functions have been ascribed to the lymphocyte from immunological carrier, tissue invader to that of haemopoietic stem cell (Porter and Cooper 1962). It is of interest that

recently Talal et al (1966) have demonstrated abnormal function of peripheral lymphocytes from Sjögren's patients both with regard to in vitro transformation and failure to develop delayed sensitivity. Studies of a similar type of lymphocytes found in the salivary glands of these patients would be of interest.

The occurrence therefore of these foci of lymphocytes in the labial glands of patients with Sjögren's syndrome would add support to Waterhouse's belief that focal sialo-adenitis is indeed a focal form of Sjögren's syndrome.

As described in Chapter 28, there is good evidence for placing Sjögren's syndrome within the group of diseases suggested as having an auto-immune basis. Serological studies would suggest that there is perhaps a stronger association between Sjögren's syndrome and the <u>non-organ specific</u> auto-immune diseases although the histological changes in salivary and lacrimal glands have similar features to chronic thyroiditis, chronic gastritis and primary adreno-cortical atrophy, all of which have <u>specific</u> auto-immune associations (Anderson et al 1965).

So far auto-antibodies to salivary gland duct cells have been demonstrated in 11 out of 19 Sjögren's patients (Bertram and Halberg 1964) but these are not tissue specific and cross react with other tissues (Anderson et al 1966). Sjögren's syndrome therefore presents features of both organ-specific and non-organ specific auto-immune disease but in the absence of specific auto-antibodies the changes in Sjögren's might still be attributable to a chronic infection.

To elucidate the question of whether Sjögren's syndrome constitutes a heterogeneous group or a single clinical entity further studies will obviously be necessary. A study in which the histological appearances of the salivary glands are compared with the incidence of salivary gland auto-antibodies in larger groups of patients with Sjögren's syndrome and other "auto-immune diseases" is clearly necessary. From the present studies it would appear that labial gland biopsy would be a suitable simple method of furthering these investigations.

CHAPTER 32

SJÖGREN'S SYNDROME AND THYROID DISEASE

Several workers have reported thyroid disease and thyroid auto-antibodies in patients with Sjögren's syndrome (Anderson et al 1961, Buchanan et al 1961, Hijmans et al 1961). The incidence of thyroglobulin antibodies has been reported as approximately 30-40% (Anderson et al 1961, Bloch and Bunim 1963, Bloch et al 1965). In the series described by Anderson et al (1961) this was significantly higher than an age and sex matched hospital control group. In the present study 6 patients (20%) had thyroglobulin auto-antibodies and all but 2 of these patients had primary hypothyroidism or Hashimoto's thyroiditis. Of the patients who had negative tests for thyroglobulin auto-antibodies, 2 had thyrotoxicosis and 1 had Hashimoto's thyroiditis. This latter patient had anti-microsomal auto-antibodies. It thus appears that there is a higher than expected frequency of auto-immune thyroiditis in patients with Sjögren's syndrome. The corollary, however, has not been evaluated, i.e. whether manifestations of Sjögren's syndrome are more common in patients with auto-immune thyroiditis - Hashimoto's thyroiditis and primary

hypothyroidism. It is of some historical interest that Hashimoto (1912) first noticed the similarity in the histological appearances of the thyroid in Hashimoto's thyroiditis and the lacrimal and salivary glands in Mikulicz's disease.

METHODS

The incidence of Sjögren's syndrome in a group of 41 patients with Hashimoto's disease and in another group of 32 patients with primary myxoedema was compared to that in a control group of 36 hospital patients. The diagnosis of Hashimoto's thyroiditis was based on the presence of a positive precipitin test for anti-thyroglobulin auto-antibodies in a euthyroid or hypothyroid patient (Buchanan et al 1961). Three of the patients with Hashimoto's thyroiditis were hypothyroid when studied, but the remaining 38 patients were receiving O.2 mg. sodium thyroxine and were euthyroid. Two of the patients with Hashimoto's thyroiditis had 'definite' rheumatoid arthritis (Ropes et al 1958). The diagnosis of spontaneous primary hypothyroidism, (hypothyroidism without a goitre) was based on the clinical and laboratory criteria described by Wayne (1960) and Wayne et al (1964). Five of the patients with primary hypothyroidism were hypothyroid

when examined, and the remaining twenty seven were receiving O.2 mg./ O.3 mg. sodium thyroxine and were euthyroid. Two of the thirty two patients with primary hypothyroidism had 'definite' rheumatoid arthritis as defined by the American Rheumatism Association criteria (Ropes et al 1958).

The hospital controls consisted of thirty six patients attending as outpatients at the clinics associated with the Royal and Western Infirmaries, Glasgow. The patients had a variety of general medical disorders, none of which, however, had any known association with thyroid disease, salivary gland disease, or auto-immune disorder.

Auto-antibodies to thyroglobulin were tested by a precipitin test using the Ouchterlony-Elekplate technique (Anderson et al 1962) and by the tanned red cell haemagglutination test, using thyroglobulin coated formalised tanned sheep red cells (Fulthorpe et al 1961). The lowest serum dilution tested in the tanned red cell haemagglutination test was 1 in 16. Auto-antibody to thyroid microsomes was measured by an indirect immuno-fluorescence technique on unfixed frozen sections of thyrotoxic thyroid slices (Holborrow et al 1959) using test serum diluted one in four in the first layer.

Examination for Sjögren's Syndrome

Sialography was performed in the one hundred and nine patients, forty one with Hashimoto's thyroiditis, thirty two with primary hypothyroidism, and thirty six control patients. The hydrostatic technique using a water soluble contrast medium (Triosil '45') was employed. A constant pressure of contrast medium entering the ducts was obtained by setting the glass container 70-90 cms. above the gland to be examined, and the contrast medium flowed in using only the force of gravity. The method is described in detail in Chapter 3. Filling phase and secretory phase films were exposed, the latter five minutes after the former. The patient was given a slice of lemon to suck between the phases.

Each patient also underwent an ophthalmic examination. Kerato-conjunctivitis sicca was diagnosed using the Schirmer 11 tear test, rose bengal staining test, and slit lamp examination for punctate and filamentary keratitis. Kerato-conjunctivitis sicca was diagnosed when the Schirmer 11 tear test was less than 5 mm at five minutes, and when there was associated strongly positive rose bengal staining of the conjunctivae and/or corneae. The medical diagnoses were not known by

the ophthalmologists and the author until after the examinations were completed.

RESULTS

Sialography: Sialographic abnormalities were classified according to the changes described by Rubin and Holt (1957) and Bloch et al (1965). The results are summarised in Table IV, 14. Abnormal sialograms were found in seven (17%) of the forty one patients with Hashimoto's thyroiditis, five (16%) of the thirty two patients with primary hypothyroidism, and in six (17%) of the thirty six hospital controls. Two patients with Hashimoto's thyroiditis showed globular sialectasis, the remaining patients having only minor abnormalities consisting of punctate sialectasis. A history of intermittent xerostomia was elicited in sixteen of the eighteen patients who had abnormal sialograms. None of the patients had clinical evidence of salivary gland enlargement, either by history or examination. None of the patients with Hashimoto's thyroiditis or with primary myxoedema who had rheumatoid arthritis, had xerostomia or abnormal sialograms.

Report of Ophthalmological Examination

The eye examinations revealed kerato-conjunctivitis sicca present in a small minority of patients in each group (Table IV, 15) The prevalence was not significantly different from that in the hospital control group (5.5%).

DISCUSSION

This study shows no increased prevalence of xerostomia or sialographic abnormalities consistent with Sjögren's syndrome affecting the parotid gland, in patients with proven thyroid disease in which circulating auto-antibodies consistent with auto-immune thyroid disease are demonstrated. On the other hand the prevalence of thyroglobulin auto-antibodies in patients with Sjögren's syndrome is increased (Anderson et al 1961; Bunim 1961; Bloch and Bunim 1963; Anderson et al 1965) and thyroglobulin auto-antibodies have also been reported with increased frequency in the connective tissue diseases, rheumatoid arthritis (Anderson et al 1961) and systemic lupus erythematosus (Anderson et al 1961; Hijmans et al 1961) all of which may be associated with established kerato-conjunctivitis sicca.

The absence of an increased prevalence of xerostomia, sialographic abnormalities, and kerato-conjunctivitis sicca in auto-immune thyroid disorder may, however, be consistent with the concept that auto-immune thyroiditis is an organ specific disorder, sharing little or no overlap with the non-organ specific auto-immune diseases. There is growing evidence that the organ specific auto-immune diseases (chronic thyroiditis and gastritis, primary adrenocortical atrophy) show familial aggregation (Hall et al 1960, Te Velde et al 1964) and familial clustering has also been recorded in the connective tissue diseases (Leonhardt 1964). It is widely believed but not proven that genetic factors are present. It may be that Sjögren's syndrome is the consequence of inheritance of both traits, and it would be desirable to obtain information on the clinical auto-immune status of the siblings of a large series of patients with Sjögren's syndrome.

CONCLUSIONS - PART IV

From these studies of the salivary gland involvement in 30 patients with clinical evidence of Sjögren's syndrome reported in Chapters 29-32 the following facts emerge.

1) The two components of the "sicca complex" - xerostomia and xerophthalmia - may arise together with similar degrees of severity or they may occur and progress separately. No well defined association is present between the onset of xerostomia or xerophthalmia and the menopause.

2) The complaint of xerostomia may indicate reduced salivary flow but also may represent only the subjective sensation of dry mouth. Xerostomia due to salivary gland involvement should therefore be confirmed by sialography or salivary flow studies.

3) There was a good correlation between sialographic appearances, salivary flow studies and oral symptoms and signs in the individual patients studied.

4) The results of labial gland biopsy in a controlled series of 8 patients suggests that this simple procedure may be of diagnostic value in Sjögren's syndrome. In the patients studied a good relationship was shown between the histological evidence of labial salivary gland involvement and clinical tests of salivary gland function. The histopathological findings would suggest that focal lymphocytic sialoadenitis is a focal form of Sjögren's syndrome.

5) While a high incidence of auto-immune thyroid disease is found in Sjögren's syndrome, no increased incidence of Sjögren's syndrome was found in patients presenting with auto-immune thyroid disease.

S U M M A R Y

Some new methods and some established ones have been applied to studies of salivary glands in health and certain disease states.

A technique of recording the pattern of salivary flow has made it possible to collect saliva samples accurately at several different but constant flow rates. The effect of varying salivary flow rate on the parotid and submandibular concentrations of electrolytes, iodide, uric acid and also the activity of carbonic anhydrase has been demonstrated and the normal ranges defined. It is important to relate the concentration of some salivary constituents, e.g. iodide, to the plasma level.

Combined quantitative and radioisotopic methods have been used to determine basic values for the metabolism of iodine in salivary glands and saliva. Measurement of the salivary specific activity after a tracer dose has the advantage that it is independent of flow rate as the ratio of stable to radioiodine is constant at different flow rates. Normal ranges have been described for salivary iodide concentration, clearance, absolute quantities secreted in unit time and saliva/plasma ratios. The salivary iodide concentrating mechanism is normal in altered states of thyroid function and also in fibrocystic disease where previously high salivary iodide levels had been reported. In Sjögren's syndrome however low saliva/plasma ratios have been found and this suggest that the salivary iodide trap may be involved in this condition.

The chemical nature of the salivary iodine has been studied and found to be almost entirely in the inorganic form in health and in some thyroid disease states. In contrast the urinary iodine, normally inorganic, contains organic iodinated compounds in thyrotoxicosis and in dehalogenase deficiency. Some advantages of the salivary specific activity method over the urinary method for the indirect measurement of the plasma inorganic iodine are demonstrated.

Anions of the VIIth periodic group include iodide, bromide and pertechnetate. Pertechnetate, like iodide, is concentrated in saliva. Simultaneous administration of isotopes ^{132}I and $^{99m}TcO_4$ allow direct comparison of salivary gland concentrating ability to be made on the same saliva sample thus eliminating the variable of flow rate. The isotope $^{99m}TcO_4$ has many advantages over isotopes of iodine as a clinical tracer and its use for radioisotopic visualisation of the salivary glands has been demonstrated for the first time.

The criteria for diagnosis of oral and salivary gland involvement in Sjogren's syndrome have been examined. The comparative value of tests of salivary gland function have been assessed in 30 patients with a clinical diagnosis of Sjögren's syndrome. The advantages of the new technique of hydrostatic sialography are demonstrated. Good correlation was shown between sialographic appearances, salivary flow

rate measurements and clinical signs and symptoms. Labial gland biopsy shows a high occurrence of focal lymphocytic infiltration in patients with Sjögren's syndrome as compared with a control series. The onset of xerostomia and xerophthalmia showed no well defined relationship to the menopause. Both these components of the 'sicca syndrome' may arise together but often they commence and progress independently. While a high incidence of auto-immune thyroid disease is found in Sjögren's syndrome, an increased incidence of Sjögren's syndrome was not found in patients presenting clinically with auto-immune thyroid disease.

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