CONCENTRATION GRADIENTS FOR INORGANIC I^{131} AND CHLORIDE IN MIXED HUMAN SALIVA [1]

By NORBERT FREINKEL [2] AND SIDNEY H. INGBAR [2]

(From the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C., and the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.)

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The ability of the thyroid gland to accumulate inorganic iodide and to maintain higher concentrations than those found in the serum has been described recently by Vanderlaan and Vanderlaan (1) and by Taurog, Chaikoff and Feller (2). This process is independent of organic iodination and can be dissociated from subsequent phases of the synthesis of thyroid hormone (3, 4).

The mechanism of the thyroidal "iodide trap" remains obscure. However, the existence of extrathyroidal sites for the selective concentration of inorganic iodide has been known for some time. In 1929, Lipschitz reported gastric juice/blood concentration ratios of 10 to 15 and saliva/blood ratios of 1.5 to 7.0 in Pavlov pouches and parotid fistulae of dogs which had been given up to twenty milligrams per kilogram of sodium iodide intravenously (5). After larger doses of iodide, gradients were reversibly depressed and the concentration of iodide in alimentary secretions progressively approached that of blood. Barkan and Leistner demonstrated that this transport mechanism was specific for inorganic iodide (6). Following the administration of an iodinated protein ("Iodtropon," *i.e.*, iodinated egg-white), they observed negligible amounts of iodide in gastric juice and saliva. Moreover, massive amounts of thyroxine did not increase alimentary concentrations of iodide beyond levels which might be anticipated from the metabolic degradation of thyroid hormone into inorganic iodide (7). The early German observations have been amply confirmed by later studies of the iodide content of saliva (8–12) and gastric juice (10, 13). However, analytical methods for stable iodide have differed widely and thereby complicated quantitative intercomparisons. Introduction of radioactive iodine obviated some of the analytical difficulties. Schiff, Stevens, Molle, Steinberg, Kumpe, and Stewart reported human gastric juice/serum gradients of I^{131} ranging from 1 to 70 and salivary/serum gradients from 7 to 700 (14). Their results are in substantial agreement with other studies employing I^{131} (15, 16, 17).

The quantitative agreement between thyroidal and salivary gradients for inorganic iodide as well as the phylogenetic and embryological similarities of these structures (18) prompted an investigation of the transport of I^{131} in mixed human saliva. Salivary concentration gradients for I^{131} have been related to simultaneously measured gradients for chloride and to 24-hour thyroidal uptakes of I^{131}. The effect of thiocyanate upon these functions has been evaluated.

METHODS

Subjects were convalescent hospital and ambulatory clinic patients, as well as normal laboratory personnel. All subjects were fasting and recumbent at the time of the observation and had abstained from smoking for at least the preceding one hour. Doses of 50 to 100 μc of carrier-free I^{131} dissolved in sterile saline were administered intravenously. Twenty to thirty minutes were allowed for equilibration. Blood specimens were collected at intervals of fifteen to thirty minutes during the following two hours. Sampling was not continued beyond the third hour since organically bound I^{131} may begin to appear at this time in the blood of patients with rapid rates of hormone manufacture (19). Collections of mixed saliva (*i.e.*, serous and mucous) were secured by standardized technique: subjects chewed $3 \times 1 \times 0.5$ cm. paraffin pledgets for five minutes and discarded all saliva collected during this period. For the next two minutes the mouth was rinsed continuously with tap water in order to remove any residual paraffin and saliva. A second five minute chewing period was then started with a fresh paraffin pledget and all of the saliva was collected in beakers. Specimens of saliva were transferred to graduated centrifuge tubes and their

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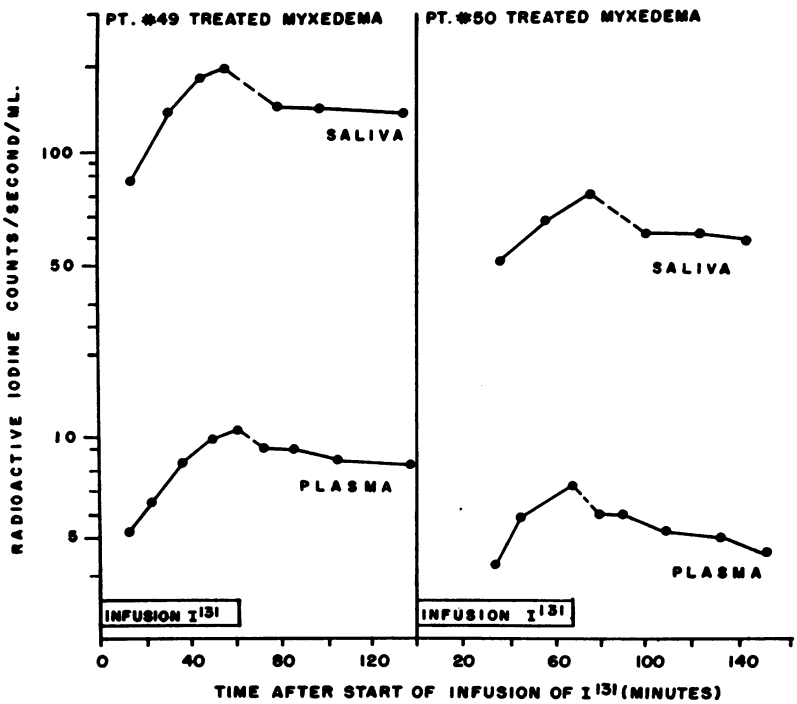
[2] Present address: Thorndike Memorial Laboratory, Boston City Hospital, Boston 18, Massachusetts.

volumes recorded following centrifugation for fifteen minutes at 3000 r.p.m. Aliquots of serum and the centrifugated saliva were pipetted into weighed planchets and one drop of alkalinized one per cent gelatin supplemented with carrier iodide was added to each. Radioactivity was measured with a thin (1.4 to 1.8 mg. per cm.^{2}) mica-end window Geiger-Mueller counter. Dried planchets were counted sufficiently long to reduce the standard deviation of the count to less than one per cent. Geiger-Mueller tube characteristics and geometry were maintained constant. A correction for self-absorption was made in the radioactivity of each sample by means of a mass-absorption curve. Chloride analyses were performed in duplicate on similar aliquots of serum and saliva by the method of Volhard as modified by Van Slyke (20).

Specimens of saliva were chromatographed on strips of Whatman No. 2 filter paper in butanol and acetic acid (21). All of the radioactivity migrated as inorganic iodide.

Saliva/serum concentration gradients for I^{131} (S/P I^{131}) and chloride (S/P Cl) were calculated from the serum concentrations of these anions observed at the midpoint of the individual salivary collection period. Following the intravenous administration of I^{131}, blood levels of radioactivity are constantly declining. Therefore, the validity of concentration gradients for iodide determined from the simultaneous measurement of salivary and plasma I^{131} rests upon two assumptions. First, equilibration of iodide between plasma and salivary cells must be instantaneous, and second, the time lapse between the elaboration of saliva and its collection must be negligible. Theoretically, any delay in transport will result in errors in the estimation of the true concentration gradient, since radioiodide appearing in the saliva at any instant would have been extracted from the plasma at some earlier time. Thus, S/P ratios obtained during rising plasma concentrations would underestimate the true gradient, whereas a falling curve could result in overestimation. Artifactual overestimation would be maximal in subjects with hyperthyroidism, in whom the most rapid disappearance of I^{131} from the blood



S/P I^{131}	16.2	17.6	18.8	19.8	15.1	15.8	16.1	10.9	9.4	11.7	10.0	10.7	10.5
FLOW(ML/MIN.)	1.58	1.64	1.70	1.33	1.72	2.03	1.68	0.45	1.42	1.17	1.01	1.72	1.78

FIG. 1. ESTIMATION OF DELAY IN SALIVARY TRANSPORT OF I^{131} BY INFUSION TECHNIQUES

In Pt. No. 50, constancy of S/P I^{131} gradients during and following infusion of I^{131} denoted negligible delay in salivary transport. In Pt. No. 49, the rising gradients during the infusion indicated a finite "lag phase" which was shown by extrapolation to be approximately seven minutes.

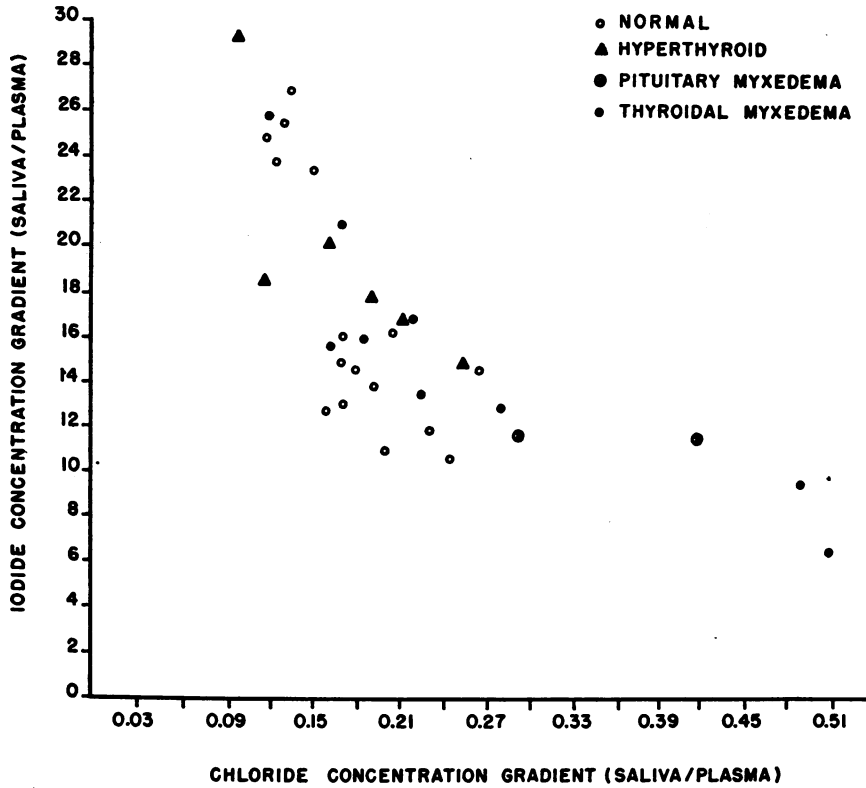


FIG. 2. THE RECIPROCAL RELATIONSHIP BETWEEN CONCENTRATION GRADIENTS FOR CHLORIDE AND IODIDE IN MIXED HUMAN SALIVA

occurs. The duration of the salivary "lag phase" may be estimated by extrapolating the simultaneous slopes of plasma and salivary radioactivity to their base line ordinates (in the case of a linear relationship) or by observing the relative positions of their peaks during ascent and descent. Such studies were performed in two athyreotic patients maintained on constant amounts of exogenous thyroid hormone. Rising plasma levels of I^{131} were produced by constant infusion; falling levels occurred after the infusion was discontinued. Specimens of plasma and saliva were collected during both periods by the methods which have been described previously. In subject No. 50, identical gradients were secured on the upslope as well as the downslope thereby indicating negligible delay in transport (Figure 1). In subject No. 49, the progressively increasing gradients during the linear infusion phase suggest a lag of approximately seven minutes (Figure 1). Correction for this delay yielded a constant S/P ratio of 20 throughout the period of rising plasma radioactivity. The depressed gradients during the post-infusion phase cannot readily be explained. Despite the crudeness of these measurements and obvious over-simplification of complex kinetics, the results are consistent with an extremely rapid, iodide transport between serum and saliva. Hence, in all subsequent studies, no correction was made for error due to delay in equilibration and/or "dead space."

Six subjects were given 1.5 to 3 grams of sodium thiocyanate intravenously. Delivery was made by a single injection over a four-minute period. Saliva/serum gradients for I^{131} and Cl were obtained prior to and following the administration of thiocyanate. The possible interference of thiocyanate in the estimation of chlorides was examined by *in vitro* recovery experiments. Addition of 0.1 to 0.5 milli-equivalents of thiocyanate to one milliliter specimens of serum or saliva containing known amounts of chloride did not interfere with chloride estimation by more than two per cent, presumably as a result of destruction of the thiocyanate during digestion of the proteins.

RESULTS

Observations were made in thirty-five individuals. All subjects who were receiving any medication which might influence thyroidal or salivary function were excluded from the present series.

The rates of disappearance of radioactivity from saliva and blood were approximately equal after the intravenous administration of I^{131}. Serial estimates of saliva/serum gradients (S/P ratios) for I^{131} and Cl were averaged for the compilation of Figure 2 and Table I. In the entire series, the

TABLE I

Metabolic activity and simultaneous concentration gradients for iodide and chloride in mixed human saliva

Subject file No.	Diagnosis	S/P I^{131}	S/P Cl	Flow (*ml./min.*)	24-hour thyroidal uptake of I^{131}
	I. Normal Metabolism				
7	Rheumatic Heart Disease	23.5	.150	0.85	33
9	Rheumatoid Arthritis	24.9	.119	2.53	12
18	Neurasthenia	16.2	.204	0.73	—
23	Rheumatoid Arthritis	27.0	.144	0.74	21
24	Rheumatoid Arthritis	14.6	.264	1.16	—
25	Nephrocalcinosis	14.6	.179	1.15	21
26	Esophageal Ulcer	21.1	.170	—	13
28	Rheumatoid Arthritis	11.9	.229	4.70	12
31	Nephrocalcinosis	13.1	.171	2.34	24
34	Laboratory Worker	25.5	.129	0.91	17
35	Neurasthenia	17.1	.160	0.65	14
36	Infectious Hepatitis	12.7	.160	—	17
39	Subacute Thyroiditis	10.9	.201	1.94	0
42	Neurasthenia	14.9	.170	0.65	18
43	Thrombophlebitis	13.8	.191	2.11	—
44	Varicose veins	10.6	.243	1.55	—
45	Pyelonephritis	23.8	.124	1.15	21
Mean		17.4	.177	1.54	
S.E.M.*		±1.4	±.010	±0.27	
	II. Hypometabolism				
3	Panhypopituitarism	11.6	.414	0.91	0
8	Surgical Myxedema†	16.8	.217	1.70	4
10	Surgical Myxedema†	13.6	.224	2.05	5
11	Surgical Myxedema†	25.8	.120	1.30	3
13	Primary Myxedema	15.7	.188	1.63	1
14	Surgical Myxedema‡	16.0	.183	1.76	0
20	Surgical Myxedema‡	12.9	.278	1.93	12
22	Panhypopituitarism	11.7	.291	1.93	2
33	Surgical Myxedema†	6.6	.506	1.46	2
38	Surgical Myxedema†	9.6	.484	0.79	4
Mean		14.0	.291	1.55	
S.E.M.		±1.6	±.042	±0.14	
	III. Hypermetabolism				
1	Thyrotoxicosis	29.4	.099	1.23	96
2	Thyrotoxicosis	20.5	.160	1.23	72
6	Thyrotoxicosis	14.8	.251	2.05	53
11	Thyrotoxicosis	16.8	.212	1.30	72
29	Thyrotoxicosis	18.9	.178	0.79	71
32	Thyrotoxicosis	18.4	.116	2.65	76
Mean		19.8	.169	1.54	
S.E.M.		±2.1	±.023	±0.28	

* Standard Error of the Mean.
† Post-operative: total thyroidectomy for carcinoma.
‡ Post-operative: subtotal thyroidectomy for hyperthyroidism.

mean S/P I^{131} was 16.8 ± 0.98;[8] S/P chloride averaged 0.210 ± .017[8] (Table I). Salivary chloride content never equalled that of serum. When concentration gradients for iodide were plotted against those which had been simultaneously determined for chloride, an inverse curvilinear relationship of high statistical significance was observed (r, − 0.74; p, less than 0.001 [22]) (Figure 2). Even greater correlation was obtained when log S/P I^{131} was plotted versus log S/P Cl (r, − 0.87).

During the test periods, mixed saliva was secreted at an average rate of 1.54 ± 0.15 ml. per min.[8] No consistent relationship was noted between rates of flow and electrolyte composition (Table I). Mixed saliva could not be collected with an accuracy comparable to that obtained in the measurement of the concentration of I^{131} and chlo-

[8] Mean ± standard error of the mean.

TABLE II

Constancy of salivary gradients

Subject file No.	Interval	S/P I^{131}	S/P Cl	Flow (*ml./min.*)
1	—	29.4	.099	1.23
	11 weeks	29.3	.117	1.16
25	—	14.6	.179	1.15
	3 weeks	12.6	.249	2.30
31	—	12.6	.111	2.20
	4 weeks	13.1	.171	2.34

ride in aliquots. Since a salivary mechanism for reabsorption of water has never been demonstrated, it is doubtful whether measurements of clearance utilizing flow values offer any more reliable estimate of osmotic work than S/P ratios.

Reproducibility of paraffin stimulation was assessed in three individuals by repeating studies at widely spaced intervals. Constancy of S/P I^{131} ratios within the same individual was seen (Table II).

Intravenous administration of 1.5 to 3.0 grams of sodium thiocyanate caused a sixty to eighty per cent reduction in salivary gradients for I^{131} (Table III). In no instance was the concentrating mechanism abolished completely; the lowest observed S/P I^{131} was 2.8. Comparable alterations in flow or chloride transport were not seen (Table III). Depression of I^{131} transport could be demonstrated within two minutes after administration of thiocyanate. In some subjects this reduction persisted at constant levels for sixty to ninety minutes (Figure 3). Prior treatment with sixty mg. of Thyrotropin [4] for five days did not appreciably modify the effectiveness of thiocyanate in subject No. 3. The promptness of thiocyanate action was viewed as additional evidence for rapid transport across salivary cells.

Correlation between the magnitude of the S/P ratios for I^{131} and the 24 hour uptake of I^{131} by the thyroid could not be demonstrated. Since salivary

[4] Thyrotropin, Armour, Lot K46808R was kindly supplied by Dr. C. J. O'Donovan of Armour Laboratories. Dosages are cited in mg. equivalent to Armour standard (2R3). Potency was approximately 0.5 U.S.P. units per mg. equivalent.

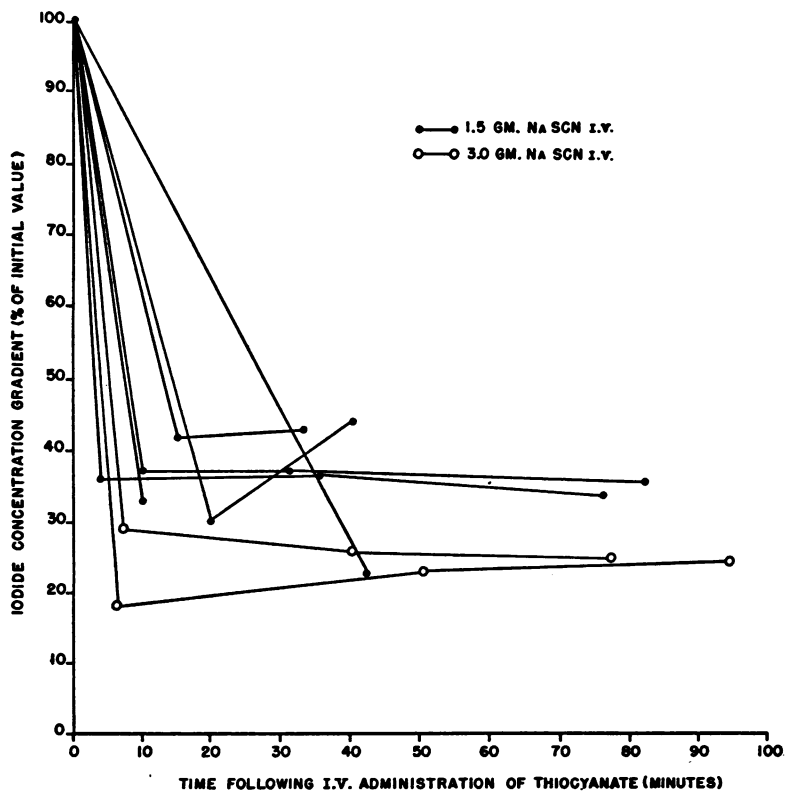


FIG. 3. DEPRESSION OF SALIVARY SECRETION OF IODIDE BY THIOCYANATE ION

TABLE III

Effect of thiocyanate upon salivary transport mechanisms

Subject file No.	Dose	S/P I^{131}		S/P Cl		Flow		Salivary Cl	
		Pre†	Post†	Pre	Post	Pre	Post	Pre	Post
	Gm.					*ml./min.*		*mEq./L.*	
3	1.5	11.6	4.1	.414	.386	0.91	0.95	42.9	40.3
3a*	1.5	8.1	3.4	.585	.565	1.47	1.12	60.7	57.2
13	1.5	15.7	5.8	.188	.197	1.63	1.37	18.2	18.9
14	1.5	16.0	3.7	.183	.202	1.76	2.18	18.1	20.0
31	1.5	13.1	4.6	.171	.143	2.34	2.08	18.8	15.7
25	1.5	12.6	4.6	.249	.239	2.30	2.16	27.6	26.4
43	3.0	10.6	2.8	.243	.297	1.55	1.66	23.8	29.1
44	3.0	13.8	3.0	.191	.233	2.11	1.93	18.4	22.2

* Subject was given 30 units of Thyrotropin, Armour, during the five days preceding the study.
† Pre and post refer to average values preceding and following the administration of thiocyanate.

gradients for iodide were maintained in two patients with untreated panhypopituitarism, in one subject with primary thyroid failure, and in seven subjects with myxedema following surgical thyroidectomy, it may be inferred that concentrating ability is operative even when production of either thyrotropin or thyroid hormone is subnormal.

DISCUSSION

Elaboration of any secretion which differs in composition from that of an ultrafiltrate of plasma entails the performance of osmotic work. Early studies of the metabolic concomitants of salivary activity disclosed that such work is accompanied by the extraction of glucose and oxygen from the perfusing blood and the addition of lactic acid, and phosphates to the venous effluent (23). Simultaneous depletion of glandular glycogen and creatine phosphate has also been described (24). Some correlation between levels of metabolism and osmotic efficiency might be expected. It is interesting, therefore, that the three individuals (subject Nos. 3, 33, and 38) who were least able to concentrate iodide or to withhold chloride were in the hypometabolic group. The remaining subjects exhibited no simple correlation between osmotic performance and metabolic activity.

An inverse relationship between salivary chloride and iodide has been demonstrated in the present studies, despite the marked disparity in their concentrations in the plasma. Such reciprocity need not denote a common mechanism for transport. In parotid saliva, chloride concentration rises with increasing rates of flow (25). It has been suggested that iodide concentration in mixed saliva increases with decreasing rates of flow (17). Therefore, the correlation between S/P ratios for chloride and I^{131} may reflect merely an inverse dependency upon flow. Even with standardized stimulus and technique of collection, this could not be demonstrated in our heterogeneous population. It is difficult to evaluate the contribution of flow to the ionic composition of mixed saliva. Concentration of iodide occurs largely in the serous component (17). Thus, admixture of varying quantities of mucus might not distort the reciprocity between concentration gradients, but might obscure their dependency upon rates of serous secretion.

Thyroidal (1), gastric (26) and renal (27) transport mechanisms may be affected by thiocyanate. In the present studies, a salivary locus of thiocyanate action has also been demonstrated. In some subjects concentration gradients for I^{131} in saliva remained constantly depressed for two to three hours following intravenous administration of thiocyanate despite the fact that levels of thiocyanate in the plasma must have undergone fivefold reduction during this interval, *i.e.*, from an initial dilution within the blood volume alone to ultimate equilibration throughout the "thiocyanate space" (28). The reduction in concentration of iodide in saliva was unaccompanied by comparable changes in the exclusion of chloride from saliva. Thus, thiocyanate produced a dissociation of the reciprocity between chloride and iodide. This would argue against a common salivary transport for chloride and iodide although the possibility cannot be excluded that there exists a multi-stage system of transport which is shared at a point not affected by thiocyanate.

To what extent salivary and thyroidal mechanisms for the accumulation of inorganic iodide are identical remains unanswered. Certainly absolute concentration gradients and extraction ratios are similar in magnitude at both sites. Furthermore, blockade by thiocyanate is equally effective in the thyroid and salivary glands. However, actual concentration of thiocyanate occurs in saliva (29) whereas there is no evidence for such a phenomenon within the thyroid (30, 31). Moreover, a well-defined relationship between thyroidal activity and salivary gradients cannot be demonstrated, although in some instances of profound hypometabolism, the osmotic efficiency of salivary glands is depressed.

SUMMARY

1. In thirty-five human subjects, salivary concentration gradients for I^{131} have been correlated with thyroidal activity and with simultaneous measurements of gradients for chloride. Mixed saliva was collected following standardized stimulation with paraffin; reproducibility of the technique was assessed by serial observations at intervals varying from minutes to weeks.

2. Infusion techniques failed to reveal any significant "lag phase" between cellular penetration and salivary elaboration of I^{131}. Saliva/serum gradients (S/P ratios) were therefore derived by concurrent estimates of plasma and salivary concentrations of chloride and I^{131}. S/P I^{131} averaged 16.8 ± 0.98; S/P chloride averaged $0.210 \pm .017$. Osmotic performance did not differ among euthyroid and hyperthyroid individuals. Three subjects with profound hypometabolism, showed diminished capacity to concentrate iodide and to withhold chloride.

3. Administration of thiocyanate produced a sixty to eighty per cent reduction in S/P I^{131} within two to six minutes, without systematic alterations in salivary flow or chloride.

4. Curvilinear reciprocity between S/P ratios for iodide and chloride could be demonstrated. The evidence is inadequate to assess whether this represented interaction of anionic transport mechanisms or merely an inverse dependency upon flow.

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