to a small volume under reduced pressure (bath temperature <35°) using a flash evaporator. When evaporation became very slow, absolute alcohol was added to aid removal of water by co-distillation. The last traces of water were removed using an oil pump fitted with a Dry Ice trap and the solid residue was dried over phosphorus pentoxide *in vacuo* at 4°.

The dry solid was triturated with absolute methanol (about 15 ml.) and removed by centrifugation. This procedure was repeated once more. The residue was transferred to a sintered glass funnel with the aid of absolute methanol and washed with this solvent until the chloride ion in the filtrate was negative (silver nitrate test). The total volume of the methanol washes was 36 ml., and it contained only 248 OD units (3% loss). The product was washed with acetone and then anhydrous ether to give a dry, amorphous, white powder—421 mg., uridine:labile P:total P; 1.00:1.00:1.97. This material was homogeneous by electrophoresis and a 50-mg. sample was recovered in 96% yield after rechromatography on a 1 × 6 cm. column of Dowex-1-chloride (single peak using elution similar to that just described). The material was active both in the P^{32} exchange assay and in polymer formation with polynucleotide phosphorylase.[3] The lithium was slightly inhibitory in the exchange assay and reduced the rate of polymer formation about 9%.

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NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF ALABAMA MEDICAL CENTER, MEDICAL COLLEGE AND SCHOOL OF DENTISTRY]

Electrophoretic and Ultracentrifugal Components of Human Salivary Secretions

BY JANE REID PATTON[1] AND WARD PIGMAN

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Electrophoretic and ultracentrifugal analyses were made on samples of individual human parotid and submaxillary secretions. Each type of secretion contained about 10 to 12 electrophoretic components, although some were minor and often did not appear in many individual secretions. Three or four components were evident in the ultracentrifuge patterns. One sample of sublingual secretion was analyzed in the ultracentrifuge and showed six components with a major 15.6S component. All secretions seemed much different from blood serum. The presence of several materials behaving as cations at *p*H 8.5 was noted. The parotid secretion also contained small amounts of several fast-moving components. The parotid and submaxillary secretions showed many similarities but were quite different from the single sample of sublingual secretion studied.

Introduction

Prior to the completion of the present work, Tiselius electrophoretic and ultracentrifugal analyses of the individual salivary secretions had not been carried out, and only two earlier studies were available for whole saliva.[2-4] Subsequently, electrophoretic data for parotid secretions have been reported by Zipkin, Adamik and Saroff[5] and by Drevon and Donikian[6] and for whole salivas and parotid secretions by Geller and Rovelstad[7] in abstract form. Amylase was identified in the electrophoretic and ultracentrifugal patterns of parotid secretion in an earlier report from this Laboratory.[8]

Paper electrophoresis has been applied to whole saliva by Kinersly and Leite[9,10] and to parotid saliva by Drevon and Donikian.[6] However, the resolution is very poor despite extensive attempts to improve it.

The present paper describes the results of Tiselius electrophoretic analyses of human parotid and submaxillary secretions at *p*H 6.0, 7.0 and 8.5 in Miller–Golder[11] buffers, ionic strength, 0.1. It also presents ultracentrifugal analyses of human parotid and submaxillary secretions and one sample of sublingual secretion. No data on whole saliva were obtained because of the complexity of the component secretions and because of the rapid changes that occurred in the viscosity and the ability to form a mucin clot.

Experimental

Human parotid and submaxillary secretions were collected separately and concentrated by dialysis to about one-fourth of their original volume by dialysis against polyvinylpyrrolidone as described earlier.[8] The solutions were then dialyzed against the buffer for 65 to 70 hr. The secretions were kept at 0 to 4° during all of these stages. The buffers were Veronal or phosphate containing considerable sodium chloride, ionic strength 0.1.[11]

Most of the electrophoretic measurements were made in a 6-cc. cell, but some were made in a 2-cc. cell using the Perkin–Elmer Model 38 Tiselius Electrophoresis Apparatus. The ultracentrifugal analyses were performed in the Spinco Model E Ultracentrifuge at about 26–32° and usually at 59,780 r.p.m. A 12 mm., 4° cell was used.

Results

Electrophoretic Components.—Satisfactory electrophoretic analyses of human parotid and submaxillary secretions could be made by the use of the Miller–Golder buffers at *p*H 6, 7 and 8.5 at ionic strength, 0.1. It was necessary to concentrate the secretions to one-third or one-fourth of their original volume and to use the 6-cc. cell. The

(1) Public Health Service predoctorate research fellow of the National Institutes of Health, 1950–1956. The work has also been supported by other NIH grants (A-216-C; A-1303-C). It represents a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Alabama, 1956.

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TABLE I

ELECTROPHORETIC COMPONENTS OF THE PAROTID GLAND SECRETION[a]

Subject	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12
					Electrophoretic mobility[b] (cm./sec./volt/cm. × 10⁵)							
					*p*H 6.0							
BM		+3.4m[c]			+1.1m		−0.6i	−2.0m	−3.9m			
BM			+2.8m		+1.9m	+0.5i	− .9i					
BM			+2.4i			+ .2p	− .6p					
FB			+3.0i		+1.4i	+ .3i	− .4p	−1.8m	−3.1i			− 8.4m
Mean		+3.4	+2.7		+1.5	+0.3	−0.6	−1.9	−3.5			− 8.4
					*p*H 7.0							
BM		+3.1m			+1.0p		−0.7p	−2.5m	−4.0m	−5.4m		
BM			+2.7i		+0.7p		−0.8p	−2.7m	−4.1m			
BM		+3.2m	+2.2m		+ .5p		−1.0p	−2.6i	−3.8m	−5.6m	−7.0m	
BM		+3.0m	+2.2i		+ .7p		−1.0p	−2.8m	−4.3m	−5.9m		− 8.6m
Mean		+3.1	+2.4		+0.7		−0.9	−2.6	−4.0	−5.6	−7.0	− 8.6
					*p*H 8.5							
BM				+0.4p								
BM		+2.6i		−0.1p			−1.5i	−3.2i	−4.5m			
BM		+2.9i	+1.7m	+0.5p		−1.4i		−3.0m	−4.7m	−6.3m		−10.4m
BM		+2.5m	+1.6i	+ .2p		−1.2p		−3.2i	−4.4m	−6.3m		−12.9m
BM		+2.5m	+1.6i	+ .1p			−1.6i	−3.1m				−11.3m
MH		+2.8m		+ .1i			−1.9p	−3.3i	−4.7m			
FB		+2.1i		+ .5p	0.0m		−1.8p	−3.2m	−4.6m		−9.3m	
FB		+2.8i		+ .8p			−1.5p	−2.7i	−4.4m	−5.8m	−8.4m	
FB		+2.7i		+ .4p			−1.8p	−3.1m	−4.8m	−6.4m	−9.2m	
FB		+2.5i		+ .8p		−1.4p		−2.9m	−4.4m	−5.9m	−8.5m	
FB	+3.2m	+2.5m		+ .4p		−1.2p		−2.5m	−4.1m	−5.7m	−8.7m	
FB		+2.7m	+1.2i	+ .6i	0.0m	−1.3p		−2.6m	−4.3m	−5.9m	−8.9m	
FB		+2.4m		+ .9i	−0.1i	−1.3p	−1.6m	−3.1i	−4.5m	−6.3m	−8.9m	
FB		+2.2m		+ .8p		−0.8m	−1.7i	−3.1m	−4.7m	−6.1m	−9.1m	
JR		+2.5i	+1.5m	0.0p			−1.6p	−2.8m	−4.5m	−5.9m	−8.9m	−11.4m
JR		+2.4m	+1.3m	+ .1i		−1.2p	−1.7i	−2.9m	−4.7m	−6.0m	−8.7m	−11.6m
WW		+2.2m		+ .3p		−1.1i	−1.7i	−3.1m	−4.7m	−6.3m	−8.6m	−12.0m
FP		+2.3m	+1.7m	+ .4p		−0.9i	−1.7i	−3.0m	−4.4m	−6.1m		−11.5m
HC		+2.3m	+0.7m	+ .2i		−1.3m	−1.7p	−3.3m	−4.6m	−6.2m		−11.9m
Mean	+3.2	+2.5	+1.4	+0.4	0.0	−1.2	−1.7	−3.0	−4.5	−6.1	−8.8	−11.6

[a] Stimulated by chewing paraffin. [b] Mobilities were calculated from the descending limb after electrophoresis in the Miller–Golder buffers of 0.1 ionic strength. [c] Relative concentration are indicated by the letters: p, principal; i, intermediate; m, minor.

TABLE II

ELECTROPHORETIC COMPONENTS[a] OF THE SUBMAXILLARY GLAND SECRETION[b]

Subject	SME1	SME2	SME3	SME4	SME5	SME6	SME7	SME8	SME9	SME10
				Mobility[c] of components (cm./sec./volt/cm. × 10^5)						
				*p*H 6.0						
JR	.....		.....	+0.6m[d]	−0.5i	−1.3i	−2.6m	−3.7i	.....	.....
BM	.....		.....	.....	.....	−1.6i	−2.9m	.....	−4.1i	.....
BM	.....		.....	.....	− .3m	−1.5i	.....	−3.8m	−4.8m	.....
FB	+6.6m		+2.2i	+ .4m	− .6p	.....	.....	−3.4i	−5.3m	−7.7m
FB	.....		+2.5m	+ .8m	− .6p	.....	.....	−3.4i	.....	.....
FB	.....		.....	+ .3m	− .9p	.....	+2.8m	−3.8m	.....	.....
Av.	+6.6		+2.4	+0.5	−0.6	−1.5	−2.8	−3.6	−4.7	−7.7
				*p*H 7.0						
BM		+3.5m	.....	+0.5m	−1.3i		−3.3i	−4.5i		
BM		.....	+1.8m	+0.4m	−1.1p		−3.1i	−4.3i		
Av.		+3.5	+1.8	+0.4	−1.2		−3.2	−4.4		
				*p*H 8.5						
LM	.....	.....	+0.1m	−1.5i		−2.8m	−4.0i	.....	.....	
BM	+3.3m	.....	+ .2m	−1.5i		−3.2i	−4.1i	.....	.....	
BM	.....	+2.0m	.....	−1.0m	−2.3i	.....	−4.4i	.....	−5.8i	
BM	.....	.....	.....	−1.0m	−2.2m	.....	−4.1i	−5.2i	.....	
BM	.....	.....	+ .4m	−1.2i		−3.3i	−4.6i	.....	.....	
BM	+4.1m	.....	+ .5m	−1.2i		−3.4i	−4.3i	.....	.....	
BM	+3.8m	.....	+ .3m	−1.3i		−3.4i	−4.3i	.....	−6.2m	
BM	+3.5m	.....	+ .1m	−1.5m		−3.6i	−4.6i	.....	.....	
Av.	+3.7	+2.0	+0.3	−1.3	−2.2	−3.3	−4.3	−5.2	−6.0	

[a] Miller-Golder buffers, 0.1 ionic strength. [b] Stimulated by swabbing the tongue approximately once a minute with an applicator dipped in 1% acetic acid solution. [c] Mobilities were calculated from the descending limb. [d] Relative concentrations are indicated by the letters: p, principal; i, intermediate and m, minor component.

use of 0.2 ionic strength buffers gave poor resolution.

Typical electrophoretic patterns are presented in Fig. 1. The mobilities of the electrophoretic components found in the descending patterns of the secretions at *p*H 6, 7 and 8.5 are listed in Tables I and II. The classification of the components at the various *p*H values was made on the basis of a plot of the mean mobility *versus* *p*H. Since all components were not present in all secretions and individual variations for mobility were evident, the classification is unavoidably somewhat arbitrary.

TABLE III

SEDIMENTATION COMPONENTS OF THE SALIVARY SECRETIONS

Subject	*p*H[a]	Sedimentation rates ($S_{20,w}$)					
		Parotid secretion[c]					
		PS_1	PS_2	PS_3	PS_4		
JR	..	1.6p[b]		4.7i			
JR	..	0.9p		4.4i			
JR	..	1.2p		4.2i			
JR	..	1.1p		4.3i	9.9m		
JR	8.5	1.4p		4.3i	14.3m		
JR	8.5	1.0p		3.8i	11.2m		
FB	6.0	1.1p	1.8p	3.9i	10.4m		
FB	6.0	1.1p	2.2p	3.8i	9.6m		
FB	8.5	1.0p	2.0p	4.3i	11.7m		
FB	8.5	1.1p	2.1p	4.3i	10.2m		
FB	8.5	1.0p	2.2p	4.3i	11.5m		
FB	8.5	1.0p	2.0p	4.2i	11.3m		
FB	8.5	0.4p	1.3p				
FB	8.5	0.8p	2.1p	3.8i	10.9m		
FB	8.5	1.1p	2.1p	4.1i			
FB	8.5	1.0p	1.7p	3.9i	11.1m		
FB	8.5	0.7p	1.1p	3.8i	9.2m		
WW	8.5	0.8p	1.5p	5.6i	14.2m		
FP	8.5	0.7p		4.0i	10.7m		
HC	8.5	1.3p		3.9i	11.0m		
HC	8.5	1.4p		4.0i	13.3m		
Mean		1.0	1.8	4.2	11.3		
		Submaxillary secretion[d]					
		SMS_1	SMS_2	SMS_3			
JR	..	1.2i		10.7m			
JR	..	1.3p		10.4m			
FB	..	1.6p		11.8m			
FB	..	1.0p		12.1m			
FB	6.0	1.0p	4.3i	10.9m			
FB	6.0	1.5p	3.9i	10.2m			
FB	6.0	1.4p	4.3i	10.8m			
FP	..	1.6p	4.0i				
Mean		1.3	4.1	11.0			
		Sublingual secretion[d]					
		SLS_1	SLS_2	SlS_3	SLS_4	SlS_5	SLS_6
FB	..	2.1m	5.2m	7.5m	11.2m	15.6p	19.5m

[a] Where no data are given, the *p*H is that of the secretion or concentrate; otherwise the *p*H is that obtained by dialysis against the appropriate Miller–Golder buffer. [b] Paraffin stimulated. [c] Relative concentrations are indicated by the letters: p, principal; i, intermediate; m, minor. [d] Stimulated by swabbing the tongue approximately once a minute with an applicator dipped in 1% acetic acid solution.

Because of the relatively large number of components present, the resolution was insufficient to justify area measurements and calculations of relative concentration. Instead, the relative prominence of the components was indicated in the tables as principal, intermediate or minor. A minor component was sometimes barely distinguishable, and its presence may be questionable.

Both types of secretions appeared to show the presence of 10 to 12 electrophoretically separable components, one of which PE_6 or PE_7 and SME_3 or SME_4 may be a salt boundary. No single secretion contained all components. The components were

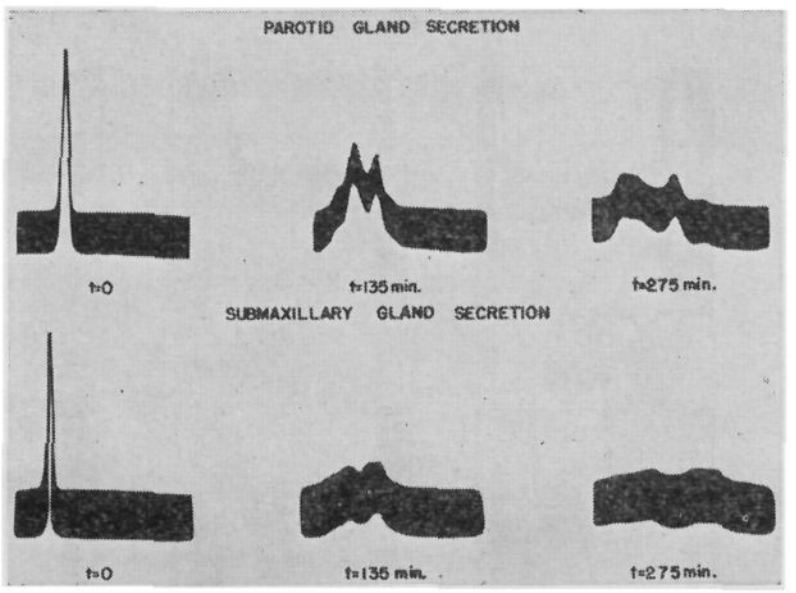

Fig. 1.—Electrophoretic patterns of salivary gland secretion; Miller–Golder buffer, μ = 0.1, *p*H 8.5, FS = 3.1 cm.

given arbitrary designation as PE_1 to PE_{12} for parotid secretions and SME_1 to SME_{10} for submaxillary secretions. The highest subscripts indicate the greatest anionic mobility.

The principal components of the parotid secretion had mobilities at *p*H 8.5 of about +0.2, −1.2, and -1.7×10^{-5} cm./sec./volt/cm. Those for the submaxillary secretion were less definite but probably can be classed as those with mobilities at *p*H 8.5 of −1.3, −3.3, and -4.3×10^{-5} cm./sec./volt/cm.

Ultracentrifugal Components.—The sedimentation behavior of several submaxillary and parotid secretions and one sample of difficultly obtainable sublingual secretion is listed in Table III. Typical patterns are presented in Fig. 2. Several different conditions of *p*H and concentration were employed with little effect except that best results were obtained for the submaxillary and parotid secretions after concentration. The sublingual secretion was sufficiently concentrated to be analyzed without additional concentration.

As many as four components were found in the parotid secretion. The principal components have sedimentation rates of 1.0 and 1.8 *S*; a less prominent component had a sedimentation rate of 4.2 *S* and a minor component had a rate of 11.3 *S*. The 1.8 and 11.3 *S* components were not present in many of the samples studied.

The three components of the submaxillary secretion had sedimentation rates of 1.3, 4.1 and 11.0 *S*, which were similar to those of the parotid secretion and showed the same relative concentrations.

The single sample of sublingual secretion was found to be more complex than the other secretions, and six components were evident in the patterns. Two components had sedimentation rates (2.1 and 11.2 *S*) similar to those of the parotid secretion, but the others were different. The rapidly sedimenting components were the principal ones; the principal peak had a sedimentation rate of 15.6 *S*.

Discussion

The parotid and submaxillary secretions from humans were found to contain 6 to 12 electrophoretically separable components at *p*H 6, 7 and 8.5 and 3 to 4 ultracentrifugal components. No single secretion contained all electrophoretic components,

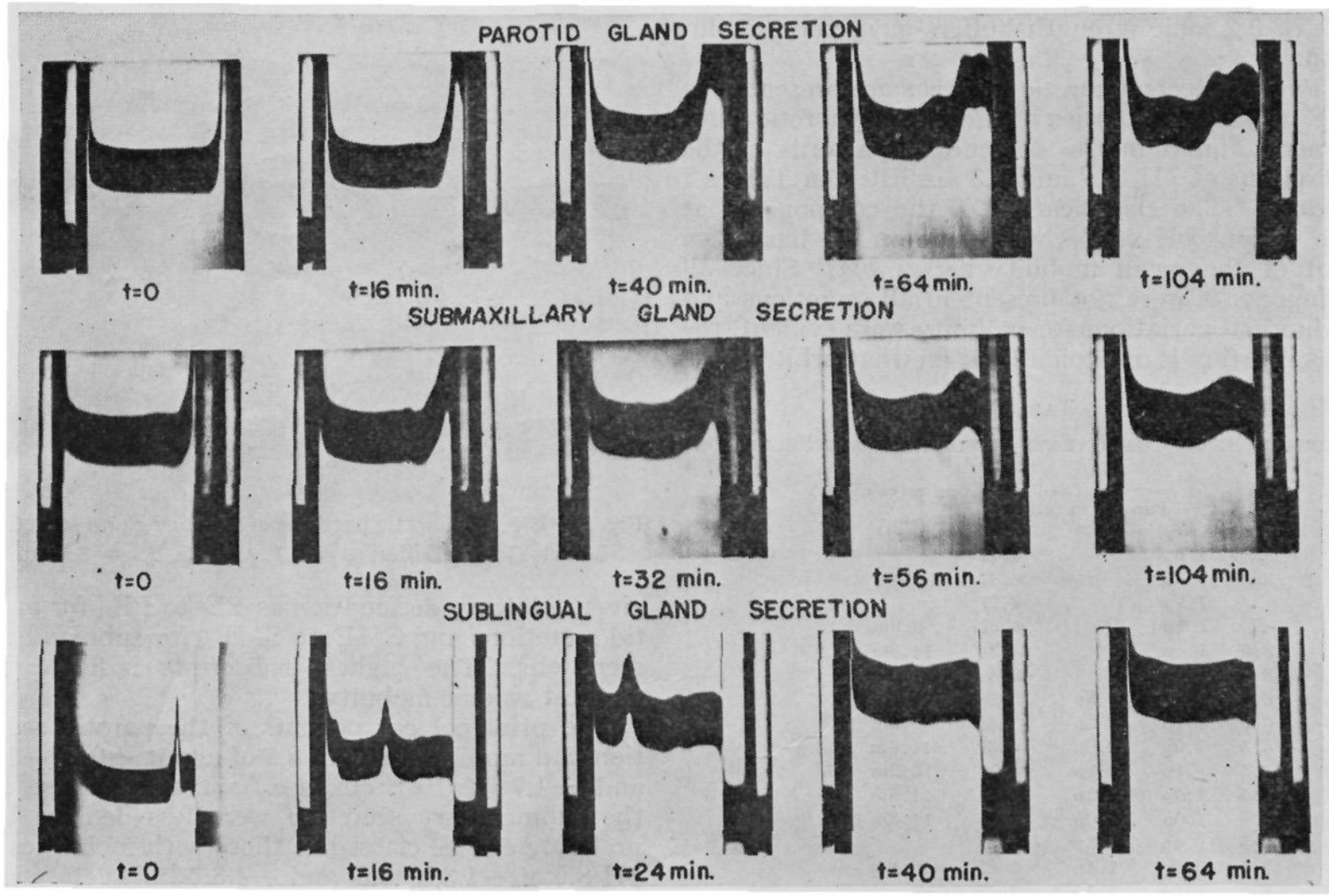

Fig. 2.—Ultracentrifugal patterns of salivary gland secretions.

and considerable differences between individual samples, especially from different persons were apparent. A single individual seemed to show more constancy in the number of electrophoretic and ultracentrifugal components in successive samples than did samples from different persons. However, the number of individuals was too small to draw definite conclusions. In any case, some of the components were scarcely evident in the patterns.

Electrophoretic analyses of human parotid saliva have been reported by Zipkin, Adamik and Saroff.[5] Results similar to those in the present study were obtained, but only seven components were reported. Although some of the mean mobilities differ as much as $\pm 0.6 \times 10^{-5}$ cm./sec./volt/cm., the seven components (I through VII) seemed to correspond to PE_2, PE_3, PE_4, PE_6, PE_7, PE_8 and PE_9, respectively.

Geller and Rovelstad[7] found components which apparently corresponded to PE_7, PE_8, PE_{10}, PE_{11}, SME_2, SME_4, SME_6, SME_7 and SME_9. Whole salivas, parotid secretion and extra-parotid saliva were studied by them.

Drevon and Donikian[6] did not list the mobilities of the four components found at *p*H 8.6 or the five at *p*H 7.15, obtained by paper electrophoresis and verified by solution electrophoresis. Two of them were said to have mobilities corresponding to albumin and γ-globulin, however, and probably are the same as PE_7 and PE_{10} found in the present study.

The reason so many more components were found in this study than in the others may be due to the fact that more samples were analyzed in the present study. In all, nine of the present parotid electrophoretic components have been confirmed by the other workers. No single sample contained all twelve of the components. The three components not found by the other workers occurred in less than half of the samples.

It was not possible to evaluate the extent of the contribution of the salt boundary to the peaks, nor to locate it exactly. It probably accounted for part of the area of PE_6 or PE_7 and SME_3 or SME_4.

The human submaxillary and parotid secretions showed marked differences from human serum, in the number and distribution of the electrophoretic and ultracentrifugal components.[12]

By immunological procedures, whole saliva has been found to contain at least four serum components but also to have a number of unique salivary proteins.[13]

Minor components of infrequent occurrence had electrophoretic mobilities close to those expected for serum albumin, PE_{10} and SME_9; the 4.2 and 4.1 *S* sedimentation components might be albumins. However, if albumin is present, the electrophoretic patterns indicate that the amount is small.

In work previously reported from this Laboratory, the amylase component of parotid secretion was identified as having an electrophoretic mobility of -1.5×10^{-5} cm./sec./volt/cm. at *p*H 8.5, under the conditions of the present work, and an $S_{20,w}$ of 4.1 *S*. This corresponds to the PE_6, PE_7 and PS_3 components and possibly to the SME_4, SME_5 and SMS_2 components of the submaxillary secretion.[8]

The presence of cations at *p*H 4.5 is a marked

(12) G. L. Miller, E. E. Miller and E. S. Eitelman, *Archiv. Biochem.*, **29**, 413 (1950).

(13) S. A. Ellison and P. A. Mashino, *J. Dent. Res.*, **37**, 28 (1958). (Abstract).

feature of the parotid and especially of the submaxillary secretion. Lysozyme is a basic protein known to be present in salivas. The PE_{11} and PE_{12} components move rapidly enough possibly to correspond to acidic polysaccharides or glycoproteins.

It is generally considered that the parotid gland has purely a serous secretion, the sublingual a mucus secretion and the submaxillary a mixed secretion. The electrophoretic and ultracentrifugal patterns of the submaxillary and parotid secretions showed many similarities. In contrast, the single sample of sublingual secretion had more numerous and generally quite different ultracentrifugal components from those of the other secretions.

The only electrophoretic or ultracentrifugal component that has been identified is the amylase of parotid saliva. The high degree of complexity of the secretions and the relative lack of knowledge of the macromolecules will require considerable additional work before the major components are identified.

BIRMINGHAM, ALABAMA

[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

6-Thiouric Acid—a Metabolite of 6-Mercaptopurine

BY TI LI LOO, MARVIN E. MICHAEL, ARTHUR J. GARCEAU AND JAMES C. REID

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6-Thiouric acid has been successfully synthesized by the direct thiation of uric acid. Its structure was established by comparison with the isomeric 2- and 8-thiouric acids synthesized unequivocally. Its identity with a specimen isolated from the urine of patients treated with 6-mercaptopurine confirms that 6-thiouric acid is one of the metabolites of 6-mercaptopurine in humans.

The metabolism of 6-mercaptopurine (6-purinethiol) has been of interest because of its clinical application in the chemotherapy of leukemia. Using S^{35}-labeled 6-mercaptopurine, Elion[1,2] stated that one of the routes of metabolic transformation of 6-mercaptopurine in humans and mice was through oxidation to 6-thiouric acid. Unfortunately Elion's papers were more or less in the form of preliminary reports and no experimental details were published. It is the purpose of this paper to report for the first time the preparation, by means of the direct thiation[3] of uric acid, and characterization of 6-thiouric acid. That the thiation did indeed take place preferentially to give the desired 6-thiouric acid was established by a comparison of the thiated product with the remaining two possible isomeric thiouric acids synthesized unequivocally. Further, the synthetic 6-thiouric acid was found to be identical with a specimen isolated from the urine of patients treated with 6-mercaptopurine, thus proving conclusively that 6-thiouric acid is one of the metabolites of 6-mercaptopurine in humans.

Experimental

Preparation of 6-Thiouric Acid.—To a solution of 2.8 g. of phosphorus pentasulfide[4] in 100 ml. of dry pyridine at its refluxing temperature was added 1.7 g. of uric acid. The mixture was refluxed under constant agitation for 24 hours, care being exercised to exclude moisture. The mixture was then cautiously poured into an equal volume of boiling water and gently boiled for about five minutes. The unchanged uric acid (about 0.5 g.) that separated upon cooling to 0° was removed by filtration and the filtrate was evaporated *in vacuo* (water aspirator) to dryness at about 50°. To the residue was added 50 ml. of a saturated solution of sodium bicarbonate and the mixture was heated to 90° for a few minutes. A second crop of unreacted uric acid (about 0.9 g.) was removed at this stage by centrifugation. The supernatant, containing principally sodium 6-thiourate in solution, was again evaporated to dryness *in vacuo* (water aspirator) at about 50°. The residue was suspended in 20 ml. of water and 5 ml. of concentrated hydrochloric acid. The crude 6-thiouric acid which separated as a brown precipitate was once again warmed to 90° with 25 ml. of a saturated sodium bicarbonate solution, and evaporated to dryness *in vacuo* at 50°. This residual material was triturated with 15 ml. of water and acidified to *p*H 7.6 with 1 *N* hydrochloric acid. The 6-thiouric acid, obtained as a brown solid, was washed with water and dried on a porous plate; yield 0.22 g., 83% based on the uric acid consumed. The final purification by means of ion-exchange chromatography is the same as the procedure described below for the purification of 6-thiouric acid isolated from patients' urine.

Pure 6-thiouric acid crystallizes from water in yellow microcrystals as a hydrate, m.p. above 300°.

Anal. Calcd. for $C_5H_4N_4O_2S.H_2O$: C, 29.70; H, 2.99. Found: C, 30.29; H, 2.79.

Drying in a vacuum desiccator gave the hemihydrate.

Anal. Calcd. for $C_5H_4N_4O_2S.0.5H_2O$: C, 31.15; H, 2.59; S, 16.64. Found: C, 30.89; H, 2.67; S, 16.91.

The anhydrous compound was obtained only after drying at 120° *in vacuo* for several hours.

Anal. Calcd for $C_5H_4N_4O_2S$: C, 32.60; H, 2.19; loss of weight (from hemihydrate to anhydrous), 4.66. Found: C, 32.39; H, 2.68; loss of weight, 4.31.

It is quite stable in boiling water; solubility at 25° about 17 mg. per liter. It gives an R_f value of 0.22 by ascending chromatography[5] on Whatman no. 1 paper, using a saturated ammonium sulfate–water–isopropyl alcohol system (79:19:2)[6]; ultraviolet absorption in aqueous media (see Fig. 1) at *p*H 2.1; λ_{max} 255 mμ, log ε 3.85; 354 mμ, log ε 4.36; at *p*H 10.9; λ_{max} 233 mμ, log ε 4.11; 343 mμ, log ε 4.20.

2-Thiouric acid, a known compound, was prepared by the fusion of 4,5-diamino-2-thiouracil[7,8] with urea according to Johns and Hogan[9]; brownish-yellow crystals, m.p. above 300°, insoluble in most solvents; R_f in the ammonium sul-

(1) G. B. Elion, S. Bieber and G. H. Hitchings, *Ann. N. Y. Acad. Sci.*, **60**, 297 (1954).

(2) L. Hamilton and G. B. Elion, *ibid.*, **60**, 304 (1954).

(3) The use of the term "thiation" to mean the direct replacement of oxygen by sulfur finds precedent in G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2138 (1947).

(4) Distilled grade, Victor Chemical Works, Chicago, Ill.

(5) It should be noted that throughout this work, the paper chromatograms were visualized under ultraviolet illumination of 253.7 mμ.

(6) A. Deutsch and R. Nilsson, *Acta Chem. Scand.*, **7**, 858 (1953).

(7) W. Traube, *Ann.*, **331**, 64 (1904).

(8) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

(9) C. O. Johns and A. G. Hogan, *J. Biol. Chem.*, **14**, 299 (1913).