Frade, I. S., Massani, Z. Z., J. Lab. Clin. Med., 1959, v53, 264.

14. Schwyzer, R., Vitamins Hormones (NY), 1960, v18, 237.

15. Barbour, B. H., Casper, A., Bartter, F. C., Clin. Res., 1962, v10, 398.

16. Scornik, O. A., Paladini, A. C., Canad. Med. Assn. J., 1964, v90, 269.

17. Brown, J. J., Davies, D. L., Lever, A. F., Robertson, J. I. S., Lancet, 1963, v2, 278.

18. Bumpus, F. M., Smeby, R. R., Page, I. H., Khairallah, P. A., Canad. Med. Assn. J., 1964, v90, 190.

19. Slater, J. D. H., Barbour, B. H., Henderson, H. H., Casper, A. G. T., Bartter, F. C., J. Clin. Invest., 1963, v42, 1504.

20. Gould, A. B., Skeggs, L. T., Kahn, J. R., J. Exp. Med., 1964, v119, 389.

21. Blaquier, P., Bohr, O. F., Hoobler, S. W., Am. J. Physiol., 1960, v198, 1148.

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Hemagglutinins of Human Parotid and Submandibular Secretions.* (30716)

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Various investigations have indicated that antibodies may be present in human and animal saliva. Salivary proteins, serologically related to plasma albumin and α, β, and γ globulins, have been identified(1-5). It has been claimed that diphtheria antitoxin(6) and agglutinins against *Brucella melitensis*(7) are present in human mixed saliva. Kraus and Konno(8), in a study of the capability of indigenous human oral bacteria to stimulate the production of antibodies, have stated that antibodies appeared in whole saliva only when they were present in serum. Utilizing parenterally administered I^{131} labelled proteins, some of which subsequently appeared in saliva, Tung and Schein(9) have concluded that proteins are transferred from blood to saliva.

These findings imply that the salivary glands may permit passage of certain serum proteins. The present effort was directed toward obtaining further knowledge as to the origin of salivary proteins.

Materials and methods. Human parotid fluid, submandibular fluid, and serum were assayed for tetanus and diphtheria antibodies using the hemagglutination procedure described by Levine *et al*(10). The cells were prepared by suspending defibrinated horse blood in 10 volumes of sterile saline, mixing and centrifuging (5 minutes at 2,000 rpm) 3 times. One milliliter of the washed, packed cells was diluted with 40 ml buffered saline (pH 7.2) to obtain a 2.5% suspension. The cells were tanned by adding one volume of 1:25,000 reagent grade tannic acid in saline to an equal volume of 2.5% red cell suspension in pH 7.2 buffer and the mixture incubated at room temperature for 10 minutes. This was followed by a 10-minute centrifugation at 1500 rpm and 2 washings with pH 7.2 buffered saline. The cells were then resuspended to 2.5% and separate portions sensitized to diphtheria and tetanus toxoids as follows: 4 volumes of pH 6.4 buffered saline containing sufficient toxoid such that the final mixture was 30 Lf per milliliter was added to one volume of 2.5% tanned cells. The incubation was at room temperature, followed by centrifugation at 1,500 rpm for 5 minutes and one washing with 1:250 normal rabbit serum in saline which had been inactivated at 56°C for 30 minutes and filtered.

The samples to be tested were also inactivated at 56°C for 30 minutes and dilutions made in saline containing 1% normal rabbit serum so that 2-fold serial dilutions would bracket the end point. The 2-fold serial dilutions in 1% NRS were performed to leave

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0.5 ml in each tube. NIH standard antitoxin was used as the standard of unitage. The diphtheria standard contained 6 units per ml and the tetanus standard contained 5 units per ml. Two-fold serial dilutions of the antitoxins in 1% NRS were made with the first tubes having a dilution of 1:384 diphtheria antitoxin and 1:1280 tetanus antitoxin. Then, 0.05 ml of the tanned, sensitized cells were added to each tube, the racks of tubes vigorously shaken in order to disperse the cells evenly and allowed to stand for 2 hours at room temperature.

The end point of each series was the tube containing the last positive agglutination and contained the same unitage per ml as the end point tube of the known standard antitoxin.

Stimulated parotid saliva was obtained using a modified Carlson-Crittenden vacuum cup(11). A submandibular collector(12) was used to obtain submandibular fluid. Blood was collected by venipuncture. All collections were undertaken at the same time of day and were made preprandially.

Samples of parotid and submandibular saliva and serum were obtained from 16 male subjects and assayed for tetanus and diphtheria antibodies. Height of serum titers was used to select 9 persons for the study. Four subjects had been recently immunized against tetanus and diphtheria and had high serum titers. Four others had not been immunized recently and had relatively low serum titers. These 8 persons were then given a booster dose of combined tetanus (1 Lf dose) and diphtheria (1 Lf dose) toxoids. The last subject had high serum titers, but did not receive toxoid and was maintained as a control. Samples of blood and saliva were then collected biweekly until the serum titer reached a peak and started to decline.

To determine if the hemagglutinins in saliva were actually tetanus and diphtheria antibodies or were a nonspecific substance, parotid and submandibular fluids and serum were tested using unsensitized tanned horse cells.

Samples of serum and saliva were then adsorbed using unsensitized tanned horse erythrocytes. Aliquots of each sample were treated twice with 0.1 ml, 0.2 ml, and 0.3 ml volumes of cells per ml of test fluid. The treated serum and saliva were also tested against tetanus and diphtheria toxoid sensitized and unsensitized tanned cells.

The stability of the salivary hemagglutinin was tested by freezing and thawing submandibular fluid. To indicate if the hemagglutination phenomenon observed with saliva was species specific or related to group specific substances, hemagglutination tests were performed, using erythrocytes from several species. Cells from the rabbit, dog, sheep and man (type AB+, O—), were tested. Also, samples of submandibular fluid from each of the nine subjects were tested against their own erythrocytes.

Results. A significant increase in the serum hemagglutinin titers occurred after administration of the toxoid (Table I). The hemagglutinin titers of parotid and submandibular saliva, however, remained constant. It was also noted that prior to administration of the toxoid all of the diphtheria and 5 of the tetanus hemagglutinin titers in the submandibular fluid were greater than the serum values. Parotid and submandibular fluids, but not serum, were able to agglutinate unsensitized horse erythrocytes. The parotid fluid had a lower hemagglutinin titer than the submandibular fluid. Parotid fluid lost its agglutination ability when diluted more than 8-fold, while hemagglutinin was still detectable in the submandibular fluid after 32,000-fold dilutions.

Parotid fluid adsorbed with 0.1 ml or more erythrocytes was unable to agglutinate unsensitized tanned cells. However, it was necessary to use the 0.3 ml volume of erythrocytes before the submandibular fluid became unreactive with unsensitized tanned cells. Parotid and submandibular fluids, after adsorption, were also unable to agglutinate tetanus and diphtheria toxoid sensitized cells. In contrast, all serum hemagglutinin (antibody) titers against toxoid-sensitized tanned cells—as low as 0.004 units—were unaltered by this adsorption procedure.

Addition of purified tetanus and diphtheria toxoids to serum and saliva produced complete neutralization of the serum hemagglutinins, but did not alter the parotid and submandibular hemagglutinin titers.

TABLE I. Serum and Saliva Hemagglutinin Titers* Before and After Administration of Tetanus and Diphtheria Toxoids.

Subject No.	Base level			Peak level		
	Serum	Submandibular fluid	Parotid fluid	Serum	Submandibular fluid	Parotid fluid
			Tetanus sensitized cells			
1	32	.5	.002	64	.5	.001
2	16	4	.002	32	4	.001
3	1	.5	.002	8	.5	.001
4	2	4	.004	16	1	.002
5	.004	8	.001	32	8	.001
6	.004	.5	.002	32	1	.001
7	.004	4	.001	2	2	<.0008
8	2	4	.001	130	4	.001
9 (control)	16	.5	.001	16	.25	.002
			Diphtheria sensitized cells			
1	.5	2	.004	2	2	.002
2	2	8	.002	16	16	.002
3	.25	2	.004	1	.25	.002
4	1	4	.008	64	4	.002
5	.016	16	.002	8	16	.002
6	.016	4	.002	32	2	.002
7	.008	16	<.0004	64	8	<.0004
8	.004	16	.001	16	16	.002
9 (control)	2	2	.001	4	1	.004

* Titers expressed as hemagglutination units per ml of test fluid.

Comparisons of saliva titers obtained with toxoid-sensitized and unsensitized tanned cells showed that the highest hemagglutinin titers were obtained with unsensitized cells, while the lowest titers were noted when tetanus-sensitized cells were employed. This indicated that the interaction of a specific antigen (tetanus and diphtheria) with the erythrocytes inhibited their agglutination by saliva. Freezing and thawing of submandibular fluid caused precipitation of an appreciable amount of the mucoprotein present in this fluid, but had no noticeable effect on the hemagglutinating activity.

Submandibular saliva was able to agglutinate cells from the rabbit, dog, sheep, and man (AB+, O−), with the titers showing minor variations according to the species tested and was able to agglutinate human cells regardless of blood type and presence or absence of the Rh factor.

Discussion. The results presented indicate that diphtheria and tetanus antibodies are not transferable from blood to pure parotid and submandibular secretions *via* the salivary glands. In addition, it would appear that the salivary glands are not capable of synthesis of these proteins. These results are consistent with the findings of Tomasi and Zigelbaum (13) who have found the salivary globulin is of the γ_1A type with a 11S sedimentation rate. The tetanus and diphtheria antibodies are of the γ_2 globulin type, predominant in blood and having a 7S sedimentation rate.

Previous investigations have demonstrated that fluid enters the oral cavity from the gingival sulcus or pocket(14,15). In addition, electrophoretic studies have shown the presence of several serum components in this crevicular fluid(16,17). More recently, it was demonstrated that the fluorescein-bound component (protein) in serum was found in the crevicular fluid and whole saliva(18). However, when pure parotid and submandibular saliva was obtained, no fluorescence was observed. The authors concluded that gingival tissue fluid contributes to the protein content of whole saliva. Many earlier immunochemical investigations have utilized whole or mixed saliva. Thus, the possibility must be considered that the salivary antibodies previously noted actually originated from the crevicular fluid.

The data obtained in the current investiga-

tion indicate that a heterogenic hemagglutinin exists in pure parotid and submandibular fluids. This is supported by the findings of Beutner *et al*(19) who observed hemagglutination reactions in extracts of rabbit salivary glands. This substance may have been responsible for the apparent specific hemagglutinins (antibodies) described in previous investigations where adequate adsorptive procedures had not been utilized.

Summary. Human parotid fluid, submandibular fluid, and serum were assayed for tetanus and diphtheria antibodies. Significant increases in serum diphtheria and tetanus antibody titers occurred subsequent to toxoid administration. The antibody titers of pure parotid and submandibular saliva, however, remained constant. Saliva appears to contain a heterogenic substance which is able to agglutinate cells from horse, rabbit, dog, sheep, and man regardless of blood type or Rh factor.

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1. Mandel, I. D., Ellison, S. A., Arch. Oral Biol., 1961, v3, 77.

2. Ellison, S. A., Mashimo, P. A., Mandel, I. D., J. Dent. Res., 1960, v39, 892.

3. Gabl, F., Egger, E., Clin. Chim. Acta, 1959, v4, 62.

4. Stoffer, H. R., Kraus, F. W., Holmes, A. C., Proc. Soc. Exp. Biol. and Med., 1962, v111, 467.

5. Leach, L. B., Wyshak, G. H., Weisberger, D., J. Dent. Res., 1963, v42, 568.

6. Sugg, J. Y., Neil, J. M., J. Immunol., 1931, v20, 463.

7. Wheatcroft, M. G., J. Dent. Res., 1957, v36, 112.

8. Kraus, F. W., Konno, J., Ann. N. Y. Acad. Sci., 1963, v106, 311.

9. Tung, E. E., Schein, A. H., J. Dent. Res., 1964, v43, 423.

10. Levine, L., Wyman, L., Broderick, E. J., Ipsen, J., J. Pediatrics, 1960, v57, 836.

11. Shannon, I. L., Prigmore, J. R., Chauncey, H. H., J. Dent. Res., 1962, v41, 778.

12. Block, P., Brottman, S., N. Y. State Dent. J., 1962, v28, 116.

13. Thomasi, T. B., Zigelbaum, S., J. Clin. Invest., 1963, v42, 1552.

14. Brill, N., Krasse, B., Acta Odont. Scand., 1958, v16, 233.

15. Brill, N., Bjorn, H., ibid., 1959, v17, 11.

16. Brill, N., Bronmestam, R., ibid., 1960, v18, 95.

17. Mann, W., Abstract of paper presented at 41st General Meeting of I.A.D.R., March, 1963, Electrophoresis of Tissue Fluid from Gingival Pockets.

18. Salkind, A., Oshrain, H. I., Mandel, I. D., Periodontics, 1963, v1, 196.

19. Beutner, E. H., Genco, R., Djanian, A. Y., Witebsky, E., Proc. Soc. Exp. Biol. and Med., 1965, v118, 893.

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Inhibition by Lipopolysaccharide of Immune Phagocytosis of Latex Particles Modified with Common Antigen of Enteric Bacteria.* (30717)

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Numerous enteric bacteria, including *Escherichia, Aerobacter, Salmonella, Shigella,* and *Proteus,* share a common antigen (CA) (1,2). In view of the role of these microorganisms in infections of both man and animals, studies of the biologic and medical significance of this antigen-antibody system are of interest, for only limited information is available to date. It was shown recently that CA antibodies opsonize enteric bacteria for phagocytosis by polymorphonuclear leukocytes(3). Investigation of the bactericidal ac-

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