cellular as well as intracellular, in the walls of veins; and, on occasion, extracellular and intracellular, in the media of arterioles and muscular arteries.

Endotoxin was also detected in polymorphonuclear leukocytes, both free in the peripheral circulation and in unusual tissue sites; for example, within vessel walls and aggregated in the sinuses of the liver. Particles of endotoxin were frequently seen free in the lumens of blood vessels; in some instances endotoxin appeared to be fixed to the intima of vessels; and masses of endotoxin occasionally completely filled a vascular lumen, most often in renal glomerular capillaries, in the adrenal medullary sinuses, and in liver sinues. Endotoxin was widely distributed throughout the reticulo-endothelial system, including histiocytes. Qualitatively and quantitatively, the distribution of endotoxin within the 3 dogs was similar.

We infer from the presence of endotoxin in blood vessel walls that endotoxin may be acting directly upon these structures to produce the peripheral vascular collapse of lethal endotoxemia.

The authors thank Dr. A. M. Pappenheimer, Jr., Dr. R. R. Porter, and Mr. Leo Levine for the horse anti-rabbit gamma globulin antiserum; Dr. Richard Sidman for assistance in problems of neuroanatomy; Dr. Helen Padykula for assistance in problems in histology; and Dr. Zelma Miller for assistance in revision of the manuscript. We are indebted to Mr. Leonard Pollard for the splendid cryostat sections.

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Received September 19, 1962. P.S.E.B.M., 1962, v111.

Immunochemical Identification of Salivary Proteins.* (27825)

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Immunochemical technics, employing antisera specific for individual human plasma proteins, have been used successfully to identify proteins in human oral fluids. Using immunoelectrophoresis, Brill and Brönnestam (1) demonstrated γ-globulin, transferrin and α_2-macroglobulin in gingival pocket fluid. Ellison, Mashimo and Mandel(2) detected albumin and γ-globulin in 10 times concentrated saliva, also by immunoelectrophoresis. Mandel and Ellison(3) similarly identified the same 2 proteins in 20 to 50 times concentrated parotid gland secretion. Kraus and Sirisinha(4) measured γ-globulin in saliva and parotid gland secretion by specific precipitation.

The present study attempts to demonstrate

* This study was supported by funds from the Veterans Administration; by contract between Office of Surgeon General, Dept. of the Army, and University of Alabama; and by grant from Nat. Inst. Dental Research, U. S. Public Health Service.

TABLE I. Electrophoretic Identities, Molecular Weights and Proportions in Plasma of 8 Plasma Proteins.*

Protein	Identity	Molecular wt	F/TP (%)†
Albumin		69,000	52–55
Orosomucoid	α_1-globulin	44,000	.5
Ceruloplasmin	α_2-globulin	150,000	.3
β-lipoprotein	β_1-globulin	1,300,000	5
Transferrin	β_1-globulin	90,000	3
β_2-macroglobulin	β_2-globulin	1,000,000	2
Fibrinogen		330,000	4
γ-globulin		160,000	11

* Data on β_2-macroglobulin from Wuhrmann and Wunderly(5); remaining data from White, *et al.* (6).

† Fraction expressed as % of total protein in plasma.

8 plasma proteins (Table I) in human saliva and parotid gland secretion by the semi-quantitative ring-precipitin technic.

Materials and methods. Collection and preparation of samples. Paraffin stimulated saliva from 6 subjects was collected at room temperature. It was immediately centrifuged. The supernatant liquids were tested both without further modification ("unconcentrated") and after concentration. Concentration was effected as follows: the liquids were freeze-dried, reconstituted with deionized distilled water (except as otherwise noted) to 20 times the original concentration, and cleared by centrifugation. Tests with indicator paper showed the pH of all saliva samples to be approximately 8.

Parotid gland secretion stimulated with lemon drops was collected from 3 of the 6 subjects by means of Curby(7) caps. These secretions were centrifuged and tested immediately.

Antisera. The following antisera† to human plasma proteins were used: anti-albumin (goat), anti-orosomucoid (goat), anti-ceruloplasmin (goat), anti-β-lipoprotein (horse), anti-transferrin (goat), anti-β_2-macroglobulin (goat), anti-fibrinogen (rabbit), and anti-γ-globulin (rabbit).

Precipitin technic. The immunochemical analysis by ring-precipitin technic was performed in small tubes (inner diameter 3-5 mm). Controls included: saliva + physiologic saline, saliva + normal animal sera, and each antiserum + physiologic saline. Similar controls, substituting serum for saliva, were included for analyses of serum. Results were estimated by the appearance of the interface after the reactants had been incubated for 3-4 hours at 25°C. Results were recorded as: 4 = heavy precipitate; 3 = slight precipitate; 2 = definite line; 1 = very faint line; 0 = no reaction.

Micro-immunoelectrophoresis. The method described by Scheidegger(8) was modified as follows: Oxoid Ionagar No. 2‡ was substituted for regular agar in coating the microscope slides. These were supported by the plexiglass baffles in the Spinco§ Model R cell powered by the Spinco RD-2 Duostat. The cell compartments were filled with veronal buffer pH 8.6, $\Gamma/2 = 0.075$. Normal human serum or 20 times concentrated pooled saliva (reconstituted with the same buffer) was placed in the wells. Current was adjusted to 4 mA per slide for 1½ to 2 hours. Each trough was filled once with one of the antisera referred to above and the slides were incubated in humidified petri dishes for 2 to 3 days at 25°C. The precipitin lines were stained with Aniline Blue Black (acid black 1).‖

Micro-double-diffusion. The technic of Crowle(9) was employed without change.

General information. All centrifugation was carried out at 4°C in a Servall¶ Model SS-1 centrifuge at 5,400 *g* for 20 minutes. Oral fluids and serum were obtained from 6 healthy subjects, 3 men and 3 women, ranging in age from 23 to 52 years. All subjects worked in the same research laboratory.

Results. Saliva and parotid secretion were analyzed for plasma proteins by precipitation with commercial antisera. The antisera were first tested for specificity by immunoelectrophoresis of normal human serum (Fig. 1). They reacted with single precipitin lines in the appropriate positions except for anti-oros-

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‡ Consolidated Laboratories, Inc., Chicago Heights, Ill.

§ Spinco Division, Beckman Instruments, Inc., Belmont, Cal.

‖ Matheson Coleman and Bell Division, The Matheson Co., Cincinnati, Ohio.

¶ Ivan Sorvall, Inc., Norwalk, Conn.

omucoid which produced 2 lines and anti-fibrinogen which did not precipitate. The meaning of the 2 lines with anti-orosomucoid is not clear, but no cross reaction has been observed (see below); fibrinogen is absent from serum by definition. The anti-fibrinogen precipitated in 1 line with normal human plasma. All antisera therefore appeared to be specific.

Confirmation of this fact was obtained by the micro-double-diffusion technic. The 2 proteins most prevalent in plasma and saliva, albumin and γ-globulin, were placed in center wells in 5-10 μg amounts. The peripheral wells were filled with each antiserum except anti-fibrinogen which was not tested. Only the homologous antisera reacted with the 2 plasma proteins; no cross reactions were observed. Similar analysis by the ring-precipitin technic gave identical results.

TABLE II. Precipitin Reactions between Serum of 6 Subjects and 8 Antisera.

Antisera	J.W.	R.J.	J.K.	H.S.	F.K.	A.H.
Anti-albumin	4*	4	4	4	4	4
Anti-orosomucoid	3	3	3	3	3	3
Anti-ceruloplasmin	3	3	3	3	3	3
Anti-β-lipoprotein	4	4	4	4	4	4
Anti-transferrin	3	3	3	3	3	3
Anti-β_2-macroglobulin	3	3	3	3	3	3
Anti-fibrinogen	0	0	0	0	0	0
Anti-γ-globulin	4	4	4	4	4	4

* For recording of results see "Precipitin technic" in *Materials and methods.*

Further control tests, using the routine precipitin technic, were made to ensure that all test subjects had the selected proteins in the serum (Table II). Each serum reacted with 7 antisera. Each subject's plasma reacted with anti-fibrinogen. Reactions did not differ noticeably among the subjects' sera but differences in degree of reaction did occur with reference to the various antisera. These differences could have been due to different potencies of the antisera, to different amounts

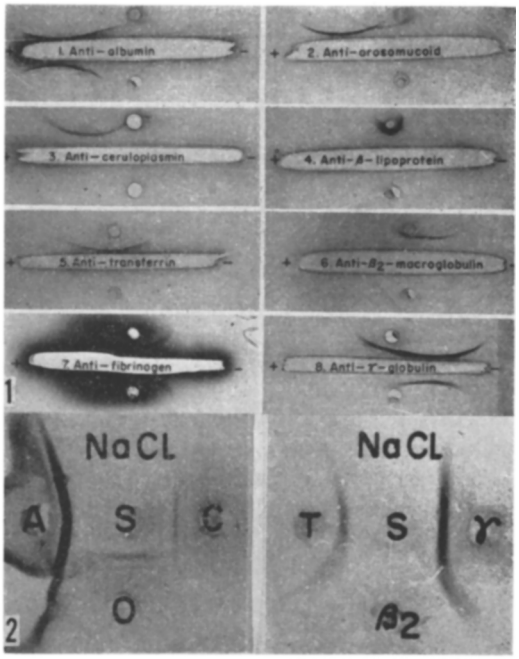

FIG. 1. Immunoelectrophoresis of serum and saliva with specific antisera. #1-6: Top well, human serum; bottom well, saliva (20 × concentrated). #5: Salivary transferrin line too faint for photographic reproduction. #7: Both wells, human plasma. #8: Top well, human serum; bottom well, saliva (20 × concentrated).

FIG. 2. Double-diffusion of saliva with specific antisera. S = saliva (20 × concentrated); NaCl = saline; A = anti-albumin; O = anti-orosomucoid; C = anti-ceruloplasmin; T = anti-transferrin; β_2 = anti-β_2-macroglobulin; γ = anti-γ-globulin.

TABLE III. Precipitin Reactions between Saliva (Concentrated and Unconcentrated) of 6 Subjects and 8 Antisera.

	J.W.		R.J.		J.K.		H.S.		F.K.		A.H.	
Antisera	C*	U*	C	U	C	U	C	U	C	U	C	U
Anti-albumin	4†	4	4	4	4	4	3	4	3	4	4	4
Anti-orosomucoid	0	2	2	2	2	1	1	1	1	1	2	2
Anti-ceruloplasmin	2	0	2	0	1	2	2	2	2	0	2	1
Anti-β-lipoprotein	0	1	2	1	1	2	2	2	2	1	2	1
Anti-transferrin	1	1	2	1	1	1	2	1	2	0	2	1
Anti-β_2-macroglobulin	2	0	0	0	0	0	1	1	1	1	0	1
Anti-fibrinogen	0	0	0	0	0	0	0	0	0	0	0	0
Anti-γ-globulin	4	4	4	4	4	4	4	4	4	4	4	4

* C, concentrated; U, unconcentrated.
† For recording of results see "Precipitin technic" in *Materials and methods.*

TABLE IV. Precipitin Reactions between Unconcentrated Parotid Secretion of 3 Subjects and 8 Antisera.

Antisera	J.W.	R.J.	J.K.
Anti-albumin	0*	1	1
Anti-orosomucoid	1	1	1
Anti-ceruloplasmin	2	2	2
Anti-β-lipoprotein	1	1	1
Anti-transferrin	1	1	1
Anti-β_2-macroglobulin	1	1	1
Anti-fibrinogen	0	0	0
Anti-γ-globulin	4	4	4

* For recording of results see "Precipitin technic" in *Materials and methods.*

of individual antigens generally available, or to both. Correspondence of heavier precipitation with greater prevalence of a plasma fraction (Table I) suggested that the degree of reaction depended on amount of antigen.

Saliva of every subject reacted with 6 of the 8 antisera (Table III), saliva of the majority of subjects reacted with anti-β_2-macroglobulin, and neither saliva nor parotid secretion (Table IV) reacted with anti-fibrinogen. The heaviest precipitation in saliva (Table III) occurred with anti-albumin and anti-γ-globulin; the weakest reaction was encountered with anti-β_2-macroglobulin. Tests with this antiserum also disclosed the only major variation among subjects.

One-half of each saliva sample had been concentrated in order to increase the amount of serologic precipitation, but the anticipated increase was observed only 10 times. In 5 of these samples the reaction would not have been detected without concentration of saliva (*i.e.*, the reaction was not apparent for the corresponding portion of unconcentrated saliva). On the other hand, 3 positive reactions occurred with unconcentrated saliva only. In most cases the degree of reaction did not differ between the 2 forms of saliva.

Important differences between the composition of parotid secretion and of saliva of the first 3 subjects (Table IV) were evident in reactions of these fluids with anti-albumin and anti-β_2-macroglobulin. Parotid fluid seemed to react with anti-albumin substantially less than saliva. The secretion gave a positive reaction with anti-β_2-macroglobulin in 2 subjects in whose saliva the reaction was undetected.

Results of the ring-precipitin study of saliva were supported by micro-immunoelectrophoresis and micro-double-diffusion. Micro-immunoelectrophoresis, applied to pooled saliva (Fig. 1), yielded 1 line each with anti-albumin, anti-transferrin and anti-γ-globulin. No reactions were observed with the antisera to orosomucoid, ceruloplasmin, β-lipoprotein, β_2-macroglobulin, and fibrinogen. Double-diffusion (Fig. 2), with pooled saliva in the center wells and antisera in the peripheral wells, revealed reactions with anti-orosomucoid and anti-ceruloplasmin which were not seen in immunoelectrophoresis.

Discussion. Specific antiserum reacts serologically with the homologous antigen. Since the 8 types of antisera used throughout the series of tests were each derived from a single lot, it follows that the antigens detected in serum or plasma, saliva and parotid fluid were immunochemically similar. The oral fluids contained proteins immunochemically similar to 7 plasma proteins.

All 7 proteins were detected in a system with a liquid-liquid interface but only 5 were capable of diffusing through a semisolid matrix. The 2 salivary proteins which were not demonstrable by diffusion through agar were β-lipoprotein and β_2-macroglobulin, the proteins having the largest molecular weights (Table I). However, molecular weight was not thought to be the explanation since these 2 proteins did migrate in the electrophoresis of serum (Fig. 1). Rather the difficulty seemed to be inherent in saliva. Similar problems are encountered whenever the resolution of saliva depends on diffusion through supporting media.

The amount of protein in saliva is very small but quantitative determination of the relatively more abundant γ-globulin is possible without preliminary concentration of saliva(4). The semiquantitative ring-precipitin technic also can be applied to unconcentrated saliva. Preliminary concentration did not generally increase the degree of precipitation. This apparent paradox may be attributed to the technic of concentrating saliva, for some insoluble material was lost in the process of reconstituting the freeze-dried supernatant fluid. This material may have

contained the proteins under consideration. However, only 3 times among 48 tests has there been evidence of complete loss of a protein by the freeze-drying procedure. Detection of the much more concentrated proteins in serum was unaffected by freeze-drying.

Albumin appeared in much lower concentration in parotid secretion than in saliva and fibrinogen was not demonstrable in either fluid. If one considers plasma to be the source of these salivary proteins, what factors may control their passage through the salivary glands and oral tissues? Evidently, molecular weight is not a controlling factor, for β_2-macroglobulin and β-lipoprotein have molecular weights 15-19 times as large as albumin and 3-4 times as large as fibrinogen (Table I). The selective permeability for albumin does not seem to be related to the shape of the molecule which is similar to the shape of most of the other protein molecules (5). Neither does it seem to consist in chemical alterations by the secretion, for the whole saliva, which contains secretion from the parotid gland, always reacted strongly with anti-albumin.

Fibrinogen has markedly different physical characteristics (asymmetry, low diffusion and high viscosity constants), low solubility and a notoriously labile constitution. Any chemical change would have to occur rapidly since fibrinogen was undetectable at the orifice of the glandular duct which is only a short distance from the blood-gland boundary. So far, the presence or absence of fibrinogen could be explained only teleologically: it is present where the blood-clotting mechanism may operate—in plasma, transudates, pleural, pericardial, and peritoneal fluids, and in lymph; it has not been reported where another clotting mechanism prevails—as in milk and colostrum; or where clotting would impede function—as in cerebrospinal fluid, synovial fluid, urine, tears, sweat, and the digestive juices including saliva.

Summary. Seven proteins in whole saliva and parotid secretion were shown to be immunochemically similar to plasma proteins by means of ring-precipitin, micro-immunoelectrophoresis and micro-double-diffusion technics employing specific antisera. By semiquantitative measurement, the differences in protein concentration among the subjects' oral fluids were small; the highest concentrations among the proteins were invariably observed for albumin and γ-globulin in saliva, and for γ-globulin in parotid secretion. Fibrinogen was the only one of 8 plasma proteins tested which could not be detected in either oral fluid.

The helpful criticism of Dr. E. E. Evans and Dr. W. J. Hammack is gratefully acknowledged.

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Received September 20, 1962. P.S.E.B.M., 1962, v111