Blood Group A,B,O(H) Activity of Urine. (27623)

HAROLD BAER AND ALLAN KLIMAN* (Introduced by M. Pittman)
Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

In the human species the A,B,O(H) blood group substances occur in many individuals in soluble form in the body fluids. Urine is the most plentiful of the body fluids but urinary blood group substances have received relatively little attention(1,2). This communication compares the blood group A,B,O(H) activity of urine with saliva.

Materials and methods. Urine and saliva samples were collected from normal individuals and patients with a variety of diseases. The urines were centrifuged at 11,900 RCF at 5°C for 10 to 30 minutes to remove cellular and particulate matter and then divided into 2 samples; one was tested for blood group activity directly and the other after concentration. For volumes of approximately 600 ml the urines were dialyzed at 5°C against several changes of 0.017% saline and then concentrated by pervaporation; the salt concentration of the concentrated urine was adjusted by dialysis against 0.85% NaCl. Small volumes were concentrated by dialysis against a 30% solution of Carbowax 20M (a product of the Union Carbide Chemicals Co.) in 0.85% NaCl. The urines, thus, were concentrated 10 to 50 times and used immediately or stored at −60°C.

Saliva samples were frozen at −60°C shortly after collection. For use they were thawed and centrifuged at 11,900 RCF at 5°C.

For the test for inhibition of hemagglutination, anti-A and anti-B blood grouping sera and *Ulex europeus* extract (anti-H) were diluted so that a macroscopic agglutination reaction of 1 to 3 large clumps was obtained. For the inhibition test a mixture of 0.1 ml of agglutinin and 0.1 ml of saliva or urine was incubated for 30 minutes at room temperature; 0.1 ml of a 3% suspension of washed erythrocytes was then added and the mixture incubated for an additional 30 minutes. Following light centrifugation and resuspension the degree of agglutination was determined by inspection with a 10× hand magnifier.

Venous blood samples in anti-coagulant were tested directly with typing sera to determine the individual's red cell type.

Results. The inhibition of anti-A and anti-B sera and *Ulex* extract by urine was specific and in agreement with the blood group of the individual (Table I). Urine and saliva gave corresponding results for the secretor status. Three specimens of fresh urine were found to give a non-specific inhibition which included inhibition of all 3 agglutinins. Following dialysis of these specimens, the non-specificity disappeared and the reactions became specific for the blood group and secretor status of the source. Table II shows the results obtained when some specimens were titrated to make a quantitative comparison of urine and saliva activity. For this purpose, the urine samples were concentrated until their anti-A inhibiting activity, in most instances, approached the activity found in saliva; the urines still failed to exhibit *Ulex* inhibiting activity.

Urine and saliva gave comparable results with *Ulex* extract (Tables I and II) when the specimens were derived from individuals of Group O. In 3 instances the urines from Group O individuals failed to inhibit when saliva did; this may have been the result of insufficient concentration. It should be noted that the urines of Group O individuals had to be concentrated to give a detectable reaction with *Ulex* extract, while in most instances, the unconcentrated urines of Group A and B individuals were capable of inhibiting anti-A and anti-B sera.

Saliva samples from group A and B secretors were capable of inhibiting the *Ulex* extract-O cell system, whereas urine samples of the same individuals (with apparently one exception) could not affect this inhibition even following concentration.

The pH of the urines, which varied from 5.2 to 7.5, did not affect the reaction. Spe-

* Present address: Blood Grouping Laboratory, Boston, Mass.

TABLE I. Comparison of the Hemagglutinin Inhibiting Activity of 54 Samples of Urine and Saliva.

	Saliva						Concentrated urine					
	Anti-A		Anti-B		*Ulex*		Anti-A		Anti-B		*Ulex*	
Blood group	Inhib.	No inhib.	Inhib.	No inhib.	Inhib.	No inhib.	Inhib.	No inhib.	Inhib.	No inhib.	Inhib.	No inhib.
A	18*	2	0	20	18	2	18	2	0	20	0	20
B	0	6	6	0	6	0	0	6	6	0	0	6
AB	2	4	2	4	1	5	2	4	2	4	0	6
O	0	0	0	0	16	6	0	0	0	0	13	9

* Numbers refer to No. of individuals from whom specimens were obtained.

TABLE II. Titration of Inhibition of Hemagglutination by Urine and Saliva.

		Anti-A			Anti-B			*Ulex*		
Name	Blood group	Urine Orig.	Urine Conc.	Saliva	Urine Orig.	Urine Conc.	Saliva	Urine Orig.	Urine Conc.	Saliva
Bae	A	μ	8*	16	—	—	—	—	—	4
Qui	A	$\mu\pm$	8	64	—	—	—	—	—	32
Til	A	μ	16	32	—	—	—	—	—	8
Del	A		16	256		—	—		—	2
Dov	A	μ	4	16	—	—	—	—	—	4
Tho	A	$\mu\pm$	4	16	—	—	—	—	—	16
Waf	O	—	—	—	—	—	—	—	2	32
Hen	O	—	—	—	—	—	—	—	8	32
And	O	—	—	—	—	—	—	—	4	64
Hea	O	—	—	—	—	—	—	—	μ	64

* Numbers are the reciprocal of the titer of blood group substance present and represent highest dilution in which activity is observed.

μ, $\mu\pm$ = complete and partial inhibition respectively with undiluted material.

cific gravity varied from 1.005 to 1.030 and urines with the lowest specific gravities did not show activity until the specimens were concentrated, but then invariably showed specific inhibition.

Discussion. The data show that the blood Group A and B activity of saliva and urine are identical but that the activity of urine is much lower. The blood Group A and B activity of urine can usually be determined directly from whole urine by inhibition of hemagglutination unless the specific gravity is extremely low. A few urines, however, exhibited non-specific inhibition before dialysis and since these were from apparently normal individuals, the cause of the difficulty is not known. Thus, if a fresh urine inhibits all 3 agglutinating systems, it is probably not from an individual of Group AB but is non-specific; retesting after dialysis should reveal the correct reaction.

The saliva of most Group A and B secretors inhibits *Ulex* extract as well as the specific antisera (Table I), but from titrations (Table II) it appears that for those specimens tested there is no direct relationship between inhibition titer of saliva for anti-A and inhibition titer for *Ulex* extract.

The failure of urine from Group A and B individuals to inhibit *Ulex* extract might merely be the reflection of a lower activity of urine. Some urines, therefore, were concentrated and titrations carried out to ascertain whether at approximately comparable anti-A inhibiting activity urine as well as saliva would exhibit *Ulex* inhibiting activity. The urines of Group A individuals with anti-A inhibiting activity close to saliva activity (Table II) still failed to inhibit the *Ulex* extract.

It appears, therefore, that there is a difference between saliva and urine of group A and B individuals that is revealed by the inhibition of *Ulex* extract. These results may merely reflect the low activity of urine for *Ulex* extract since even urine from Group O individuals required concentration to exhibit

this activity (Table II). However, some data suggest that urinary blood group substance is either degraded or of smaller molecular size than salivary blood group substance(2,3) and such altered molecules may conceivably have altered activities.

Summary. The blood group activity in human urine has been investigated by means of inhibition of hemagglutination and the results compared to the activity of saliva. Blood Group A and B activity was the same when measured in saliva or urine, although a few urine specimens showed non-specific inhibition unless dialyzed. *Ulex* extract inhibiting activity was present in saliva specimens of secretors from all blood groups, whereas in urine, this activity could not be demonstrated except in specimens obtained from Group O individuals. *Ulex* extract is not useful, therefore, as a reagent for determining the secretor status of an individual when urine is the source of antigen.

We wish to acknowledge the valuable technical assistance of Mr. Leroy Samuels.

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Received May 24, 1962. P.S.E.B.M., 1962, v110.

Pituitary Gonadotrophic and Growth Hormone Levels in Sheep Plasma.* (27624)

E. F. ELLINGTON,† A. N. CONTOPOULOS AND M. T. CLEGG

Department of Animal Husbandry, University of California, Davis, and Department of Anatomy, San Francisco Medical Center, University of California

Considerable effort has been expended on the development of methods for quantitation of pituitary hormone levels in blood. Although there have been some studies reporting gonadotrophic activity in the blood of small non-pregnant laboratory animals such as the gonadectomized rat(1,2,3,4), the estrous rabbit(5), and the mouse(6), the extensive literature, recently reviewed(7,8), is concerned predominantly with investigations concerning levels of these hormones in human blood.

With regard to blood gonadotrophic activity in the larger non-pregnant mammalian species, very little information is available; such activity has been demonstrated in the estrous pig(9), and in the non-pregnant mare (10). Rimington and Rowlands(11) could not confirm the presence of hormone in the blood of the non-pregnant mare; and Santolucito(12) could not detect circulating gonadotrophic hormones in sheep blood during various stages of the estrous cycle. Reports demonstrating the presence of growth hormone activity in the blood of non-human mammalian species are similarly rare. Gemzell *et al.*(13) reported values of 0.57 μg of growth hormone per ml in a calf, whereas Trenkle *et al.*(14) found values ranging from 5 to 74 μg of growth hormone per 100 ml of serum from non-pregnant and non-lactating sexually mature cows.

Recently a simple technic has been developed(15) for collection of blood from the cavernous sinus of sheep. Inasmuch as the cavernous sinus is one of the main avenues for venous drainage of the pituitary, the opportunity to detect pituitary hormones in blood is considerably enhanced; the concentration of these substances should be higher in blood obtained from the cavernous sinus. The following study was undertaken to compare gonadotrophic and growth hormone activity in blood plasma from the cavernous sinus as compared with levels in blood obtained from jugular or recurrent tarsal veins.

Materials and methods. Samples of plasma

* Supported in part by U.S.P.H.S. grants and grant-in-aid from Syntex Animal Products.

† NIH Predoctoral Fellow.