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Received October 8, 1962. P.S.E.B.M., 1963, v112.

Agglutinogens and Isoagglutinins in Caries-Immune and Susceptible Human Salivas.* (27976)

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Putkonen(1) reported the presence of isoagglutinins and isoagglutinogens in human saliva. Many authors have since reported the presence of blood group substances (agglutinogens) in human saliva and other body fluids. Schiff and Sasaki(2) found that ability to secrete blood group substances is dependent on the Mendelian Law. More recently, Moharram(3) reported the presence of group specific agglutinins in human saliva but did not discuss it in detail.

The purpose of this study was to compare dental caries-immune and susceptible individuals (as classified by Weisenstein and Green)(4) according to blood type, ability to secrete agglutinogens, and ability to secrete isoagglutinins. It was considered that this work might indicate if immunity to caries is linked to any blood group characteristics.

Materials and methods. Whole paraffin-stimulated saliva was collected from 12 caries-immune and 16 caries-susceptible individuals and parotid saliva from some of each group. Blood types were determined by the slide test technic. Whole saliva was placed in the refrigerator after collection allowing the mucin to precipitate. The saliva was then centrifuged and the supernatant divided into 2 portions, one of which was boiled, and both were frozen. Parotid saliva was collected with a modified Carlson-Crittenden device(5) and immediately frozen.

Agglutinogens. Secretors of agglutinogens were separated from non-secretors by the *Ulex europeus* seed extract technic described by Boyd and Shapleigh(6). One-tenth ml of whole stimulated boiled saliva of various dilutions was mixed with 0.1 ml (2 units) of *Ulex* extract in 75 × 8 mm test tubes. (A 1:16 dilution of *Ulex* extract usually caused agglutination of type O RBC's; a 1:8 was considered 2 agglutinating units.) The tubes were incubated for 30 min at room temperature and 0.1 ml of washed 1% type O RBC's was added and mixed. After 1 hr the tubes were examined for hemagglutination inhibition. All hemagglutination inhibition tests were read by the Salk pattern method and then gently tapped to detect very strong agglutination which forms a button resembling a negative Salk pattern. Inhibition was considered to have occurred only when both methods were negative.

Secretion of A and B agglutinogens was checked in a similar manner using anti-A and anti-B blood grouping sera. Those individuals found to be secretors by the *Ulex* method and showing no A or B agglutinogens were considered to be type O secretors.

Isoagglutinins. A combination of hemagglutination and hemagglutination inhibition technics was used to detect isoagglutinins in saliva. One-tenth ml of saliva (not boiled)

* This investigation was supported by a grant to Henry Ford Hospital by Procter and Gamble Co., Cincinnati, and by grant from Nat. Inst. for Dental Research.

TABLE I. Blood Groups of Caries-Immune and Susceptible Individuals.

IMMUNES

ABO type	Rh type CDE	CDe	cDE	cDe	cde	Total	Frequency† of dist in Det.	Expected No.	X^2 value and probability
O	3	3	1			7	42.9	6.4	*
A				1	1	2	39.2	5.9	$X^2 = 4.25$ $P = <.05$
B	1	1				2	13.3	1.9	*
AB		1	1	2		4	4.8	.7	$X^2 = 18.91$ $P = <.01$
Total	4	5	2	3	1				

SUSCEPTIBLES

ABO type	CDE	CDe	cDE	cDe	cde	Total	Frequency† of dist in Det.	Expected No.	X^2 value and probability
O	2	3	2		1	8	42.9	7.3	*
A	3	4	1		1	9	39.2	6.7	*
B						0	13.3	2.3	$X^2 = 2.66$ $P = >.10$
AB						0	4.8	.8	*
Total	5	7	3	0	2				

* These differences between observed and expected numbers were not great and were not analyzed.

† Frequency of A, B, O blood groups of Caucasians and Negros in Detroit, Mich. area as provided by American Red Cross.

was placed in 4 test tubes containing 0.1 ml of washed 1% type A RBC's, 4 tubes containing type B, and 1 tube of type O. They were incubated at 37°C for 30 min, then washed twice by centrifugation with 0.5 ml of saline. After all but about 0.1 ml of the supernatant had been poured off from the second centrifugation, the tubes were gently tapped to detect agglutination. The unagglutinated tubes then received 0.1 ml of 4 dilutions of blood grouping sera, anti-A added to the tubes of saliva mixed with type A RBC's and anti-B added to the tubes of saliva mixed with type B RBC's. The saliva mixed with type O RBC's received 0.1 ml of saline and served as a control. Dilutions of anti-sera were made to contain 0, 1, 2, and 3 agglutinating units. The corresponding dilutions were usually 1:800, 400, 200, and 100. The tubes were shaken, allowed to stand until a Salk pattern was formed, then recorded and gently tapped to detect very strong agglutination.

Hemagglutination and hemagglutination inhibition tests with dilutions of saliva were also done. The method was essentially the same; the saliva was diluted 1:1, 2, 4, and 8 and the anti-sera were used at 2 agglutinating units.

Results. It can be seen in Table I that all four blood groups and both Rh groups were found in caries-immune individuals. A large number of type AB immunes (26.6%) and a small number of type A immunes (13.3%) were found. The probabilities of these occurring in a random sample is less than 0.01 and 0.05 respectively, as estimated by the chi square method. Blood groups B and AB were not found in the susceptible group. By the chi square method, this is not statistically significant.

Secretors and non-secretors of agglutinogens were found in both immune and susceptible groups (Table II), demonstrating a wide range of titers. Nearly 91% of the immunes were secretors and 82.4% of the susceptibles were secretors. Results of others (3,8) show the normal occurrence of secretors to be 70 to 80%. The "t" test showed no significant differences.

Isoagglutinin secretors (Table III) were found in both groups. All of the immune group except the type AB individuals were secretors, 100% of those which might be capable of secretion. Only 31.3% of the susceptibles were secretors. The "t" test showed this difference to be significant.

Immune individual J.T. and suceptible

individual E. S. in Table III, although blood type O, showed the presence of only isoagglutinin B in their salivas. Repeated tests gave the same results. It can not now be determined whether these individuals do not produce type A isoagglutinins or whether their secretion is too minimal to be detected by the methods used.

TABLE II. Agglutinogens in Caries-Immune and Susceptible Salivas.

Immunes				Susceptibles			
Subject	Blood type	Secretion of agglutinogen	Titer	Subject	Blood type	Secretion of agglutinogen	Titer
JT	O+	O	128	JC	O+	O	128
YB	"	"	128	ES	"	"	128
WD	"	"	128	CM	"	"	64
MJ	"	"	32	NC	"	"	32
NR	"	"	8	JW	"	"	1
DO	"	–	–	MF	"	–	–
DH	B+	B	1	SC	O—	O	128
JB	AB+	A, B	1024, 1024	RW	A+	A	1024
MD	"	"	64, 16	AR	"	"	1024
SS	"	"	4, 4	EB	"	"	1024
BS	"	"	4, 4	JV	"	"	1024
				SL	"	"	256
				DJ	"	"	64
				NW	"	–	–
				PW	"	–	–
				AS	A—	A	1024

	Observed No.	Expected[3, 8] No.		Observed No.	Expected[3, 8] No.
Secretors	10	8.8	Secretors	13	12.8
Non-secretors	1	2.2	Non-secretors	3	3.2
Total	11	"t" = .905 P = >.2	Total	16	

TABLE III. Isoagglutinins in Whole Caries-Immune and Susceptible Salivas.

Immunes				Susceptibles			
		Agglutinins				Agglutinins	
Subject	Blood type	Anti A	Anti B	Subject	Blood type	Anti A	Anti B
YB	O+	4+	4+	MF	O+	2+	2+
MJ	"	4+	4+	NC	"	2+	2+
JT	"	–	4+	ES	"	–	2+
WD	"	2+	2+	JC	"	–	–
NR	"	2+	2+	JW	"	–	–
DO	"	1+	1+	SC	O—	–	–
KT	A+	–	2+	CM	"	–	–
DH	B+	2+	–	EB	A+	–	4+
MD*	AB+	–	–	DJ	"	–	2+
JB*	"	–	–	RW	"	–	–
SS*	"	–	–	AR	"	–	–
BS*	"	–	–	JV	"	–	–
				SL	"	–	–
				NW	"	–	–
				PW	"	–	–
				AS	A—	–	–

	Observed No.	Expected† No.		Observed No.	%
Secretors	8	2.48	Secretors	5	31.25
Non-secretors	0	5.52	Non-secretors	11	68.75
Total	8	"t" = 4.192 P = <.001	Total	16	

* These individuals would not be expected to secrete isoagglutinins and can not be considered non-secretors.

† Values found for the susceptible group were accepted as a normal distribution.

A number of parotid saliva samples were also checked for presence of agglutinogens and isoagglutinins. No agglutinogens were detected in the parotid salivas tested, which agrees with the findings of Rex-Kiss(7). Isoagglutinins were found in the parotid salivas of those individuals who possessed them in whole saliva, but not in parotid salivas of those who showed none in whole saliva.

Isoagglutinins, which appeared to be incomplete or blocking type, were found with the agglutination inhibition test in both immune and susceptible salivas. The titer was always low, never greater than 1:2, and not always consistently found in the same individuals. The possibility of receptor destroying enzymes being responsible for agglutination inhibition, rather than incomplete isoagglutinins, was checked. Using 8 samples, supernatants from a mixture of saliva (0.2 ml) and RBC's (0.1 ml) were mixed with fresh RBC's and after incubation one and 2 units of anti-sera added. Agglutination inhibition was not detected.

Discussion. This limited study shows a proportionately large percentage of blood type AB and a small percentage of type A, in caries-immunes, compared to a normal distribution. Although these differences are statistically significant, the importance of these findings should not be judged until a larger number of immunes is studied. The large number of caries-immunes who were secretors of isoagglutinins also is significant and further studies may show that this secretory ability, as well as other blood group characteristics, could be genetically associated with immunity to caries. Putkonen(1) reported the presence of isoagglutinins in salivas of only 30.9% of type O individuals. In contrast to this, 100% of the type O immunes showed isoagglutinins and only 42.9% of the susceptibles.

The presence of incomplete isoagglutinins cannot be evaluated until a better and more consistent method is found. It is possible that the different batches of erythrocytes used could account for variation in results. For example, if the erythrocytes used were type A_2 and the saliva being tested contained anti-A_1, agglutination may not occur, or so weakly as to be detected only by the agglutination inhibition technic. The presence of agglutinogens in saliva sometimes interfered with the agglutination inhibition test. This occurred most often with high titer type A agglutinogen secretors. Type A agglutinogen may have been associated with the RBC's even after washing and then could have combined with the diluted commercial Type A anti-serum to inhibit agglutination, producing what first appeared to be incomplete Type A agglutinins in saliva of Type A individuals. It should be noted that the possibility of receptor destroying enzymes existing in saliva is not supported by the evidence in this study.

Several of the whole saliva specimens showed a brownish discoloration. Hemoglobin was suspected but not found in any specimens by scanning on a Coleman Jr. spectrophotometer at wave lengths from 400 to 550 mμ and comparing to a standard.

The findings reported are suggestive, and indicate the desirability of further work on the physiological differences between caries-immune and susceptible human beings.

Summary. Paraffin-stimulated whole saliva and parotid saliva was collected from 12 caries-immune and 16 caries-susceptible individuals. Blood types and Rh factors were determined, and presence of agglutinogens and isoagglutinins in their salivas was investigated. This study showed differences between the 2 types of individuals, the most outstanding of which was the unusually high number of isoagglutinin secretors in the type O caries-immune group. In the immune group the occurrence of blood type AB was higher, and type A lower than a normal distribution. Agglutinogen secretors were normally distributed in both groups.

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Received October 8, 1962. P.S.E.B.M., 1963, v112.

Respiratory Enzyme Activities in Different Regions of the Mouse Heart.* (27977)

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The different work-loads of the chambers of the heart have led numerous investigators to study the oxygen consumption of the various parts. Three different results have been obtained in rat heart. First, oxygen consumption of the left ventricle was found by Goh and Dallam to be 25% greater than the atrium (1). Second, Olson *et al.* found that oxygen consumption of the rat ventricle was only 70% that of the atria(2), verifying the earlier results of Muus and co-workers(3). Third, Ullrick and Whitehorn found that oxygen consumption of atria and ventricles was the same (4).

To our knowledge the only study comparing the enzymatic activity of different myocardial tissues was by Cooper who found that succinic dehydrogenase activity of either ventricle was about twice that of the atria(5).

It was our belief that a more detailed study of the activities of electron transporting enzymes, such as the cytochrome *c* reductases and cytochrome oxidase, in different regions of the heart might aid in clarifying the conflicting results reported on oxygen consumption of the atria and ventricles. Information would be also obtained on the possible alternative pathways of electron transport in different myocardial tissues.

Materials and methods. Tissue samples were obtained from male mice (C57BL/6J strain, Jackson Memorial Laboratory) which had been reared on a commercial diet and were 59 to 80 days old at time of sacrifice. The mice were killed by separating manually their cervical vertebrae followed by decapitation. The hearts were removed quickly and dissected with the aid of a dissecting microscope in such a manner that each sample was composed of a maximal amount of myocardium, typical of a particular chamber wall, and a minimal amount of connective tissue. In this way, samples of paired atria, right ventricle, left ventricle, and inter-ventricular septum from the same heart were obtained.

For determination of enzymatic activity a sample was homogenized in 0.25 M sucrose using a loose-fitting Tenbroeck homogenizer. Samples were prepared and kept at 0-4°C until analyzed. Each sample was assayed for the different enzymatic activities within 2 hours after preparation. The cytochrome *c* reductases were determined spectrophotometrically at 23-25°C using the method of Lehman and Nason(6). To measure the different reductases, reduced nicotinamide-adenine dinucleotide (NADH), reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or sodium succinate was added, and the rate of cytochrome *c* reduction was determined at 550 mμ. The validity of the reductase assays was examined by adding sodium amobarbital and antimycin A to homogenates of atria and of left ventricle. NADH-cytochrome *c* reductase activity was inhibited by both of these reagents, and succino-cytochrome *c* reductase by only antimycin A, as found in other heart preparations(7). Cytochrome oxidase activity was measured by the method of Cooperstein and Lazarow(8) with cyanide controls for each sample. A unit of both reductase and oxidase activity was defined as a change of 0.001 optical density unit at 550 mμ per

* This work has been supported by grants from the National Institutes of Health, USPHS, and from the National Science Foundation.