material cannot be made at this time except to state that the material appears to be particulate in the general sense and is probably not intact RNA available to digestion with pancreatic RNASE.

The findings emphasize again that tolerance of allogeneic skin is probably not a unique phenomenon, but is essentially the same as immunologic paralysis and protein antigen overloading.

Summary. Subcellular fractions of C57BL/1 adult male mouse spleen were prepared by homogenization and centrifugation, and administered to prospective skin graft recipients, adult female C57BL/1 animals. Two fractions induced tolerance in a majority of the animals: a particulate fraction and a microsome fraction. Neither "Tris" extraction of the particulate fraction nor RNASE treatment of the microsome fraction affected the rate of tolerance induction significantly.

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Genetic Aspects of Salivary Secretion of Isoagglutinins.* (29095)

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In a previous study where an association between immunity to human dental caries and blood group characteristics was sought, an unusually large number of salivary isoagglutinin secretors were found among caries-immune individuals(1). That prompted this study of the probability of genetic control over the secretion of salivary isoagglutinins. Other investigators(2-7) have observed isoagglutinins in human saliva. Furuhata *et al* (4) have postulated genetic control by a pair of recessive genes, however Prokop(5, 6) found salivary isoagglutinins much more frequently in blood type O individuals and rejected Furuhata's hypothesis.

Materials and methods. Two hundred and one humans were used in this study, of whom 60 were selected from families in which immunity was not evident, 81 were related distantly to a caries-immune (first cousin or more distant) and a group of 60 caries-immunes and their close blood relatives. This last group was used only in mating studies because of the published association of caries-immunity and isoagglutinin secretion.

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TABLE I. Isoagglutinin Secretors Found in 141 Individuals in Blood Groups A, B, and O.

	A	B	O
	(%)	(%)	(%)
♂	9 (31)	6 (42.9)	18 (81.8)
♀	13 (31)	6 (50)	15 (68.1)
Total	22 (31)	12 (46.2)	33 (75)

TABLE II. Types of Isoagglutinins in Salivas of Blood Group O Individuals.

Study	Salivary secretions			
	Anti-A & B	Anti-A only	Anti-B only	Total
Wilson & Green	36	7	16	59
Sondermeier & Flatow	52	4	5	61

Blood types were determined by the slide method with commercial antisera. Salivary isoagglutinins were detected in whole paraffin-stimulated saliva by hemagglutination; 0.2 ml of clarified saliva was placed in Kahn tubes containing 0.1 ml of saline and 0.1 ml of washed 1% erythrocytes added. The first tube was considered to have a titer of 1:1, dilutions of saliva were made in the 0.2 ml volume. The tubes were incubated for 15 minutes at room temperature and centrifuged at 1-2000 RPM for 1-2 minutes. The tubes were tapped gently to detect agglutination. If the saliva agglutinated type A erythrocytes, anti-A was considered present, anti-B if type B cells were agglutinated. Type O erythrocytes were used as controls to detect non-specific agglutination. Cross-reacting anti-C agglutinin was detected by adsorbing saliva with A and B cells separately, then examining the supernatants after agglutination with heterologous cells(7). Serum and salivary isoagglutinin titers were determined on 19 isoagglutinin secretors and the sera of 14 non-secretors.

Results. Table I shows percentages of isoagglutinin secretors found in A, B, and O blood types and the sexes. The differences between male and female secretors were not statistically significant. There was no difference in percentage of secretors in the group of persons including relatives and the unrelated group. The highest percentage of secretors (75%) was found in type O individuals as compared to A (31%) and B (46.2%). Thirty-six type O individuals secreted both anti-A and B, 7 anti-A only, and 16 anti-B only as shown in Table II. These results are compared to those of Sondermeier and Flatow(7) in the same Table. Anti-C (cross-reacting) agglutinins were readily detected in a number of the type O persons.

Table III shows serum and salivary isoagglutinin titers of 19 isoagglutinin secretors and serum titers of 14 non-secretors. The lowest serum titer (reciprocal) of the secretors was 32 and the highest serum titer of a non-secretor was 256. Among the secretors,

TABLE III. Serum and Salivary Isoagglutinin Titers (Reciprocals).

Blood type	No. of subjects	Serum titer		Salivary titer	
		Anti-A	Anti-B	Anti-A	Anti-B
		Salivary isoagglutinin secretors (individual titers)			
A	2	0,0	32,64	0,0	4,4
	2	0,0	128,128	0,0	1,8
	2*	0,0	>2048,>2048	0,0	>16,>16
B	2	64,64	0,0	1,1	0,0
	1	128	0	1	0
O	3	32,64,64	32,32,64	0,1,2	2,2,2
	2	64,128	512,512	0,0	8,8
	2	256,256	128,256	1,4	4,4
	2	256,256	512,512	0,8	8,8
	1*	1024	2048	4	8
		Salivary isoagglutinin non-secretors (range of titers)			
A	5	0	4–128	0	0
B	5	16–64	0	0	0
O	4	64–128	16–256	0	0

* Blood donors stimulated with blood group antigen.

TABLE IV. Isoagglutinin Secretor Ability and Blood Types of Parents and Offspring.

Matings and blood types	No.	Offspring: Sec A	Sec B	Sec O	NS A	NS B	NS O
NS × NS							
A × A	4	9*		1*	5		
A × O	4	2		1	4		1
Sec × NS							
O × O	2			4			1†
A × O	2				3		1
B × O	1			2			
A × B	1			1	1		1
Sec × Sec							
O × O	4			6			1‡
B × O	1		1	1			1
A × B	1	2					
Type AB matings							
AB × A (NS)	1		1		2		
AB × A (Sec)	1	1					
Total	22						

Sec = isoagglutinin secretors.
NS = non-secretors.
* Excludes dominant inheritance.
† Male offspring, mother = secretor, father = non-secretor, excludes sex-linked recessive inheritance.
‡ Non-secretor offspring of secretor parents, one parent secreted anti-A only and the other anti-B only.

subjects with higher serum titers tended to exhibit higher salivary titers. However, it is evident that the level of serum isoagglutinins does not appear to account for secretion of isoagglutinins in the saliva.

Within the 16 families studied 22 matings suitable for examination were found. Table IV shows the combinations of isoagglutinin secretion ability and blood types of parents and offspring. Four suggestions regarding inheritance of secretor ability may be obtained from these data. 1: The matings in which both parents were type A and non-secretors produced 10 secretors and 5 non-secretor offspring; the presence of the 10 secretor offspring (single asterisk) excludes dominant inheritance of this characteristic in type A individuals. 2: A non-secretor male offspring (double asterisk) from a mating in which the father was a type O non-secretor and the mother a type O secretor excludes sex-linked recessive inheritance in type O individuals. 3: The matings in which both parents were type O and secretors produced 6 secretors and one non-secretor offspring; the presence of the latter (triple asterisk), from a mating in which one parent secreted only anti-A and the other only anti-B isoagglutinin, does not exclude any method of inheritance but suggests the presence of more than one pair of genes. 4: The matings in which one parent was type AB produced 2 secretor offspring; this suggests that the gene for secretion of isoagglutinin can be carried by type AB individuals.

Discussion. In agreement with the results of Prokop(5,6) a greater percentage of isoagglutinin secretors was found in blood group O. The simple recessive inheritance theory of Furuhata's(4) cannot account for the high percentage of secretors in group O individuals nor the type O individuals who secrete only anti-A or only anti-B. Another complexity of the inheritance of this characteristic is the presence of a cross-reacting isoagglutinin (anti-C) which is often found in type O individuals. Sondermeier and Flatow (7) detected the presence of anti-C isoagglutinin in saliva by adsorbing with erythrocytes. Their observations have been confirmed in our laboratory. Assuming genetic control over isoagglutinin secretion, the presence of 3 isoagglutinins in saliva suggests the possibility of 3 or more pairs of genes.

In the group of isoagglutinin secretors, high serum titers generally corresponded with high salivary titers; however, the data show that serum titer is not the sole factor in salivary secretion. If one considers that serum and salivary levels of isoagglutinin may be genetically independent characteristics, this does not imply that high serum titers could not produce a spill over of isoagglutinins into saliva. The lack of complete correspondence of serum and salivary levels suggests that the genetic mechanism for salivary secretion may operate on the salivary glands to effect selective filtration, or alteration of serum proteins by salivary enzymes, or local manufacture of isoagglutinins.

The matings (Table IV) are not numerous enough positively to suggest a genetic scheme for inheritance of the ability to secrete isoagglutinins. A considerable number of variables increase complexity. Blood type A,

B, and O individuals show a considerable difference in the frequency of secretors suggesting a different genetic mechanism for each blood type. Type O individuals can secrete one or more types of isoagglutinins. Type AB individuals, although unable to secrete, can produce secretor offspring. This suggests the possibility that type A and B individuals may carry genes for secretion of isoagglutinin of their own blood type, which, although not expressed in them, may be passed to their offspring. Not enough matings have been observed to indicate a method of inheritance of the secretor ability of type B individuals. The data, however, suggest that type A (anti-B) secretors inherit this ability through a pair of recessive genes and type O individuals do not inherit the secretor ability through a sex-linked recessive system. The data also suggest that observations on more matings, especially from type B individuals, will show that the ability to secrete isoagglutinin is inherited as well as the responsible mechanisms.

Furuhata(4) suggested use of the symbols V,v for isoagglutinin secretion. We do not concur with this designation because these symbols had been previously assigned to a blood factor of the "African" type by De Natale *et al*(8). In view of the common use of "secretor" for persons who secrete water-soluble blood type antigens of the A B O group in saliva, the term "iso-secretor" could be used to indicate those humans who secrete isoagglutinins in saliva. This term may be conveniently abbreviated to "iso-se." The particular isoagglutinin that they secrete can be included, as an example iso-se-A for one who secretes anti-A.

Summary. Whole saliva was collected from 201 individuals, a number of which were selected specifically for mating studies. Blood types and ability to secrete salivary isoagglutinins were determined. Salivary isoagglutinins were detected in 31% of blood type A individuals, 46.2% of type B and 75% of type O. The following observations suggest that the ability to secrete salivary isoagglutinin is genetically influenced. 1. Blood type O individuals were able to secrete anti-A without secreting anti-B isoagglutinin, or the converse. 2. A comparison of salivary and serum isoagglutinin shows that the serum titers are generally reflected by salivary titers in secretors; serum levels alone, however, do not account for the secretion of salivary isoagglutinins. 3. Twenty-two matings suggest that the ability to secrete isoagglutinin by type A humans (anti-B) is a recessive characteristic and the secretor ability in type O is not sex-linked. The term "iso-secretor" is suggested for humans who secrete isoagglutinins to avoid confusion with the term "secretor" used to describe those who secrete the A B O blood group antigens.

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