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### A, B, AND H GROUP SPECIFIC SUBSTANCES IN CERUMEN

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The sweat glands in various areas of the skin have unusual arrangements and functions. An example of this would be the glands of the external auditory meatus which produce cerumen. Cerumen is a brown substance, waxy, with a bitter taste which serves as a protective device against insects and dehydration. Cerumen is composed of two secretions—one fron the sebaceous glands and the other from the tubular coiled cerumenous glands in the skin of the meatus.<sup>1</sup>

The following paper describes the application of the absorption elution method to cerumen and the results of the use of this method with 100 experimental samples.

#### PROCEDURE

- 1. Approximately four drops of saline are added to a Kimble shell vial.
- 2. A swab moistened with saline is used to collect a sample of cerumen from the external auditory meatus of each individual.
- 3. The swab is then agitated in the saline to form an extract.
- 4. With a capillary pipette, a drop of the extract is placed in three separate wells of double-welled slides. The slides are then placed on a temperature block for 45 minutes at 55 degrees centigrade and are then removed and left to cool.
- 5. One drop of Anti-A, Anti-B, and Anti-H, respectively, are placed in the wells. Absorption is allowed to take place in a moisture chamber at four degrees centigrade for four hours.
- 6. The slides are removed and are washed free of anti-serum using approximately 125 milliliters of cold saline added directly to each well from a polyethylene wash bottle. The wells are then blotted dry and one drop of saline is placed on each of the three wells. The slides are then placed in a moisture chamber and are put on a temperature block at 55
- <sup>1</sup> MAXIMOW, ALEXANDER A. AND BLOOM, WILLIAM, A TEXTBOOK OF HISTOLOGY (W. B. Saunders Company Publisher, 1948).

degrees centigrade for ten minutes to allow the absorbed antibody to elute.

- 7. The slides are then removed from the chamber. Fresh  $A_1$  and B indicator red cells and Group O enzyme treated cells (bromelin) of approximately 0.5% are added to the respective wells. The bromelin reagent is prepared by adding 0.5 g. of concentrated bromelin powder to 90 cc of saline and 10 cc of Sorenson's Buffer. Sorenson's Buffer may be prepared as follows:
  - (1) 2.04 g. KH<sub>2</sub>PO<sub>4</sub> + 100 cc distilled H<sub>2</sub>O<sub>5</sub>
  - (2) 2.13 g. Na<sub>2</sub>HPO<sub>4</sub> + 100 cc distilled H<sub>2</sub>O.

Add 95 cc of solution (1) to 5 cc of solution (2).<sup>2</sup> An equal amount of bromelin and saline is used with the Group O cell suspension.

8. The slides are again placed in a moisture chamber and put on a rotator. Results are read at 15, 30, and 60 minutes with a microscope.

#### RESULTS

Strong indicative results were obtained from Group A, B, AB, and O secretors as well as non-secretors in each group. Of the 100 experimental samples, all were grouped according to the secretor, non-secretor status of the individual obtained from prior grouping of saliva samples. No results of secretor groupings were obtained from non-secretors and, likewise, the results show no grouping of non-secretor from a secretor sample. Several samples were repeated as many as twenty times with accurate reproducible results.

Table 1 illustrates the results obtained by using the Absorption Elution method for the grouping of cerumen with 49 A, B, O, or AB secretors. 51 nonsecretors, that is 13 type O, 15 type A, 10 type AB

<sup>2</sup> VITULIO, LOUIS R. Use of Enzyme-Treated Cells in Grouping Dried Bloodstains, JOURNAL OF CRIMINAL LAW, CRIMINOLOGY, AND POLICE SCIENCE, 57, No. 3, September 1966.

Table 1

Table 1			
_	Absorption Elution Results		
STAIN	Anti-A Se- rum A Cells	Anti-B Se- rum B Cells	Anti-H Se- rum O Cells
A secretor	++	<b> </b>	_
B secretor	ļ —	++	
B secretor	l —	++	
A secretor	+++		_
O secretor		<b>—</b>	++
AB secretor	+++	++	
O secretor	_	_	+++
AB secretor	++	++	<b>—</b>
A secretor	++	_	_
O secretor		_	++
B secretor	_	++	-
B secretor	_	+++	_
A secretor	+++		_
O secretor	_		+++
AB secretor	+++	+++	
AB secretor	+++	+++	_
O secretor		-	++
A secretor	++		
A secretor	+++	-	_
A secretor	+++	-	_
B secretor	-	+++	_
AB secretor	++	++	-
O secretor	-	- 1	+++
A secretor	+++	-	
O secretor	-	]	+++
O secretor	. — .	- 1	+++
A secretor	+++		
B secretor	- 1	+++	-
B secretor		++	. <del>.</del> .
O secretor	. <del>.</del> .		+++
A secretor	+++		<del></del> .
O secretor		- 1	+++
AB secretor	++	++	_
AB secretor	+++	+++	_
A secretor	++		
AB secretor B secretor	++	++	_
O secretor		+++	
A secretor	1	_	++
O secretor	++	_	<del>-</del> ,
AB secretor	++	+++	+++
A secretor		TTT	
A secretor	+++	_	_
O secretor	TTT	_	<u> </u>
A secretor	+++		TTT
B secretor	444	+++	_
AB secretor			_
O secretor	+++	+++	<u> </u>
AB secretor	++	_ ++	TTT
111 30010001	TT	TT	_

<sup>+++</sup> Very large clumps—only a few free cells
++ Large clumps—having more free cells
+ Smaller clumps—many free cells
- No agglutination

and 13 type B non-secretors were also tested and no agglutination resulted in all cases for anti-A serum A cells, anti-B serum B cells, and anti-H serum O cells.

#### Discussion

It is of prime importance that the sample is concentrated. There must be an adequate amount of cerumen present in order to detect the group specific substances. The swab must show the presence of the brown waxy substance in order to insure a sufficiently concentrated extract. The extract itself will be very cloudy and appear as a dispersion. The absorption elution method is shown to be satisfactory in testing samples of cerumen both in experimental cases and case work.

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