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ABH SECRETOR STATUS OF CLEFT LIP/PALATE PATIENTS

BY

RICHARD LEE BAKER

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL  
OF LOYOLA UNIVERSITY IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

JUNE

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## LIFE

Richard Lee Baker was born in Joplin, Missouri on February 20, 1939. He graduated from Joplin Senior High School in May, 1957. He obtained an Associate of Arts degree from Joplin Junior College in 1959 and a Bachelor of Arts degree from Kansas State College at Pittsburg in 1961.

Following college he enrolled at the University of Missouri at Kansas City, School of Dentistry and received the degree of Doctor of Dental Surgery in June 1964.

After two years of service with the United States Navy Dental Corps, he enrolled as a graduate student in the Department of Anatomy at the University of Tennessee in Memphis.

He practiced dentistry privately in Missouri and Texas until he enrolled in the graduate school of Orthodontics at Loyola University, Chicago, Illinois in June, 1968.

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Without the assistance and cooperation of such an interested and learned advisory board, I am certain that this study would not have been nearly so successful nor would this have been such an enlightening experience for me.

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CHAPTER I  
INTRODUCTION

A. Introductory Remarks and Statement of the Problem

The relationship of group specific substances of human body fluids to various abnormalities became a specialized form of research about three decades ago with the finding by Landsteiner and Weiner (1940) that iso-sensitization of the blood of an expectant mother to a blood factor of fetal blood cells resulted in erythroblastosis fetalis.

This dramatic explanation of the etiology of erythroblastosis renewed interest in the possibilities that other diseases and pathological phenomena are genetically inter-related to various blood factors or other group specific substances of body fluids.

While a review shows extensive investigations into blood groups and disease relationships have been accomplished, it is noted that little work has been done concerning the presence of group specific substances of saliva and the occurrence of congenital abnormalities.

Over seventy-five percent of human beings have ABH blood group substances corresponding to those of their red cells present in their saliva. The presence or absence of

these water soluble antigens A, B, or H in saliva divide humanity into two groups: secretors and non-secretors.

One of the most severe congenital conditions that Orthodontics has to deal with is cleft lip/palate. The purpose of this research is to investigate the possible existence of a relationship between ABH secretor status and cleft lip/palate condition.

#### B. Review of the Literature

The study of heredity has progressed in a little more than a century from Mendel's symbolic representation of inheritance in pea plants (1865) to an interpretation at the molecular level, related to the chemical processes involved in growth and maturation. The mechanism of heredity is now seen as a remarkable unifying feature of all kinds of living things. Significant advances serving to explain this mechanism have included Morgan (1933) and his discoveries on hereditary functions of the chromosomes, Lederberg and Beadle (1941) with their investigations of genetic mechanisms and discovery of how genes transmit hereditary characteristics and the elucidation of the three-dimensional molecular structure of DNA by Crick, Watson, and Wilkins (1962).

These principles of inheritance have been shown to fully apply to the blood groups discovered by Landsteiner (1900).



The work of Yamakami (1926) on the presence of A and B antigens in secretions and the investigations of Lehrs (1930) and Putkonen (1930) on saliva established that the presence of blood group substances were not limited to the red cells.

In 1932 Sasaki found that group specific substance of all blood types may either be present in large amounts in saliva or entirely lacking. Schiff and Sasaki (1932) found that the ability to secrete the group substances of types O, A and B is inheritable and is dependent on the Mendelian factors S and s. Friedenreich and Hartmann also confirmed that the secretion of these substances was a genetically determined trait that obeyed the Mendelian law of inheritance (1938).

Schiff (1940) stated that the saliva of a so-called "secretor" contained one of the blood group specific substances A or B, in accord with the blood group characteristic of the individual. In a "non-secretor" the group specific substance corresponding to the blood group in the red cells was absent from the saliva. The occurrence of secretors and non-secretors was studied in two groups of whites and a group of Negroes. Statistically, the difference between the

two white groups was negligible, whereas that between the Negroes and each of the white groups was definitely significant.

Weiner and Belkin (1943) tested a series of thirty pairs of saliva samples from mothers and their new-born infants for their content of group specific substances. The distinction between secretors and non-secretors was as sharp in the infants as in the mothers, indicating that this characteristic is fully developed at birth.

The heredity of the ABO system and the secretor/non-secretor system must be considered as established according to Andresen (1961). The ABO and secretor/non-secretor systems are now considered an entity in which all antigens are chemically related, but in which independent systems of genes determine the phenotypes.

Present evidence of blood groups and the relationship to disease concludes that persons belonging to different blood groups differ in their susceptibility to certain diseases of adult life.

Important and significant information about racial differences has been accumulated from studies based on the blood group systems by Mourant (1954).

Weiner (1947) suggested that investigating the possible relationship between iso-sensitization and congenital malformations would be fruitful. This suggestion was supported by reports in the literature of high incidence of congenital malformations among erythroblastotic infants.

Most of the work with blood groups and group specific substances has dealt with anomalies or disease of the gastrointestinal tract. The classical work that has encouraged many recent investigations in this field is the work of Aird in 1953. He reported a correlation between cancer of the stomach and a disturbance in the frequency of types in the ABO blood groups. The data showed the frequency of blood Group O to be less in patients with cancer of the stomach than in the general population.

Other diseases such as essential hypertension, ulcerative colitis, diabetes mellitus and rheumatic fever have been studied by various workers and compared to ABO blood group frequencies. A frequency of Group O was found in males with diabetes mellitus. Hypertension and rheumatic fever essentially showed no difference to the normal population.

The relationship between blood groups and osteogenesis imperfecta was studied in Swedish families by Smars (1961) and no significant association or linkage relation was found.

Buckwalter (1956) studied a varied group of congenital anomalies and tested the group for blood types. The malformations included those of nervous systems, cardiovascular, respiratory, genitourinary, and gastrointestinal system. Inspection of the combined data for all the anomalies, indicated no statistically significant differences existed between the anomalies and the individual blood types. The study of specific groups of anomalies also failed to show evidence of statistically significant differences.

The increasing frequency of the appearance of cleft palate and lip among newborn infants in Poland and the lack of concise explanation as to the etiology, prompted Handzel and Nowakowski (1961) to make a study of the possible relationship between the blood groups, Rh factor and the incidence of these defects. Blood groups of children demonstrating these defects and blood groups of their mothers were determined. Rh factor for both mother and child and frequently blood of both parents was typed. The conclusion drawn from Handzel's study was that no relationship was

shown between development of cleft palate and blood groups or Rhesus factor of the child.

Investigation by Roberts (1957) demonstrated that the genetically determined character of salivary ABH non-secretion is associated with duodenal ulcer. Clarke, Evans, McConnell, and Sheppard (1959) showed that non-secretors of Group O are particularly likely to develop peptic ulcers.

In a study by Dodge (1967) secretor status appeared to be unrelated to the occurrence of pyloric stenosis.

Considerable literature has developed attempting to associate blood groups and secretions of the body tissues with increased susceptibility to various diseases. The report by Aird in 1953 linking cancer of the stomach with blood group A has been corroborated, reviewed, then refuted, and even denied by different authors. Other associations such as group O with duodenal ulcer, group A with pernicious anemia and group A with diabetes mellitus, and non-secretors with duodenal ulcer have been statistically supported and as frequently contested.

## CHAPTER II

### METHODS AND MATERIALS

#### A. Selection of Subjects

Subjects for this investigation were selected from patients at Fantus Cleft Palate Clinic, Cook County Hospital and Loyola University, School of Dentistry.

The test group studied included both males and females of the Caucasian, Negro, Oriental and Indian races, who exhibited a cleft lip/palate deformity. The clefts were present in any combination and to any degree of severity. A similar control group was used, comprised of subjects exhibiting no cleft lip/palate condition.

Saliva samples were obtained and prepared in the following manner.

1. The subject was asked to rinse the mouth with water before collection of the saliva. A clean 15 ml. size test tube was used to collect about 5 ml. of saliva.

2. The specimen was transferred to another 15 ml. size test tube and heated in a boiling water bath for 10 to 15 minutes to inactivate enzymes that destroy the blood group substances.

3. The specimen was centrifuged at 5,000 rpm for five minutes.

4. The clear supernatant was transferred to another clean 15 ml. size test tube. Because it was desirable to test all specimens at one time, the supernatant samples were frozen until testing could be performed.

#### B. Method of Saliva Testing

It has been found that an anti-H agglutinin, prepared by simple saline extraction of seeds of *Ulex europaeus*, is suitable for the routine separation of individuals into secretors and non-secretors, merely by testing the ability of the subjects to inhibit the agglutination of group O erythrocytes by the undiluted *Ulex* extract. The procedure has the advantage of requiring only one reagent for saliva from individuals of any blood group, and only one type of erythrocytes for testing.

The following inhibition test procedure was used to determine the secretor or non-secretor status of each of the test and control subjects.

1. Into two properly marked 10 x 75 mm. tubes were added 1 drop of saliva (test) and 1 drop of 0.9% saline (control) respectively.

2. One drop of anti-H Lectin was added to each tube.
3. The mixture was incubated at room temperature for ten minutes.
4. One drop of a freshly three times washed 2% suspension of group O cells in 0.9% NaCl solution was added to the test mixture.
5. The mixture was incubated at room temperature for ten minutes.
6. The tube was centrifuged at 3,400 rpm for 30 seconds.
7. The cells were gently resuspended and placed on a clean glass slide and read for the presence or absence of agglutination.

Interpretation of the inhibition test for ABH secretor status is outlined below.

#### Secretor

The group O cells in the tube containing saliva are not agglutinated indicating that the anti-H has been neutralized by H substance in the saliva.

#### Non-Secretor

The group O cells in the tube containing saliva are agglutinated, indicating the absence of H substance in the saliva.



Control

The group O cells in the control tube are agglutinated.

## CHAPTER III

### FINDINGS

Table I contains the secretor/non-secretor reaction status for each member of the experimental and control sample. All subjects are listed according to their incidence of appearance by selection.

Table II indicates the statistical evaluation of secretor/non-secretor frequency of occurrence measured by the Chi-square ( $X^2$ ) Test.

Table I  
Secretor/Non-Secretor Reaction Status of  
Experimental and Control Samples

+ = secretor      - = non-secretor

Experimental Subjects  
(Cleft Lip/Palate Patients)

1.	D.B.	+
2.	B.W.	+
3.	B.W.	-
4.	B.M.	+
5.	B.J.	+
6.	D.L.	+
7.	D.C.	-
8.	D.T.	+
9.	H.T.	+
10.	R.M.	+
11.	R.W.	+
12.	L.H.	-
13.	R.T.	+
14.	L.S.	+
15.	M.G.	+
16.	C.I.	+
17.	H.K.	+
18.	A.A.	+
19.	L.G.	+
20.	M.Z.	-
21.	M.T.	+
22.	E.H.	-
23.	G.B.	+
24.	M.G.	+
25.	D.E.	+
26.	E.W.	+

21 Secretors  
5 Non-Secretors

Control Subjects

1.	K.J.	-
2.	J.S.	+
3.	J.P.	+
4.	D.F.	-
5.	J.P.	+
6.	D.P.	+
7.	E.B.	+
8.	T.N.	-
9.	J.C.	+
10.	J.L.	+
11.	R.W.	+
12.	R.F.	+
13.	J.D.	+
14.	N.C.	+
15.	K.S.	-
16.	D.S.	+
17.	A.C.	+
18.	B.S.	-
19.	P.H.	+
20.	M.M.	-
21.	P.K.	+
22.	M.F.	+
23.	N.B.	+
24.	J.S.	+
25.	G.T.	-
26.	J.S.	+

19 Secretors  
7 Non-Secretors

Table II

Secretor/Non-Secretor Chi-Square ( $\chi^2$ )Evaluation of Significance

The Chi-square formula is employed to determine if any statistically significant frequency of occurrence of secretors/non-secretors exists.

$$\chi^2 = \sum \frac{(\text{Observed number} - \text{Expected number})^2}{\text{Expected number}}$$

$$\chi^2 = \sum \frac{(21 - 19)^2}{19} + \frac{(5 - 7)^2}{7}$$

$$\chi^2 = 0.782$$

The value .782 represents a significance level of approximately 47%. A significance level of 5% and below is considered to be meaningful and merit further investigation.

## CHAPTER IV

### DISCUSSION

The findings as revealed in Table I and Table II demonstrate that no significant difference of secretor/non-secretor incidence exists between the cleft lip/palate patients and the control subjects in this experiment.

While the primary purpose of this investigation was to determine if any significant relationship existed between cleft lip/palate and control incidence of secretor/non-secretor ability, it seems appropriate to comment on the frequency of ABH secretors and non-secretors according to population.

Frequency distinctions between secretors and non-secretors are difficult to make. Before the utilization of Ulex the published figures for Europe varied with different workers. Further, North American Indians and Australian aborigines differ widely from the Europeans; in thirty Blood Indian families from Alberta all the parents were secretors, and in 120 aborigines all but four were secretors.

Probably the best series of random people classified for ABH secretion is found in the controls of the Liverpool peptic ulcer investigations. Secretor tests on the saliva

of 1,118 random "control" persons showed 77.28% secretors and 22.72% non-secretors. Other studies show that the ability to produce a water soluble substance of ABH specificity in the saliva is present in 75 to 80 percent of humans.

Findings in this investigation disclose 73.0 percent secretors and 27.0 percent non-secretors in the control group. 80.7 percent secretors and 19.3 percent non-secretors were found in the cleft lip/palate test group.

## CHAPTER V

### SUMMARY AND CONCLUSIONS

#### A. Summary

A research project was undertaken to investigate the possible existence of a relationship between the ABH saliva secretor status and cleft lip/palate subjects. A control group of similar subjects lacking the cleft lip/palate abnormality was used. Both the test and control groups were composed of twenty-six subjects. The individuals tested were male and female of the Caucasian, Negro, Oriental and Indian races.

The values of incidence were put to the mathematical Chi-square test to show the significance of correlation. The statistics obtained showed that when the test and control groups were compared to one another, they did not differ greatly and a significance level of approximately 47 percent existed as compared to the standard 5 percent level of significance.

#### B. Conclusions

The conclusions that can be stated from the findings in the experiment are the following. From this investigation it appears the incidence of cleft lip/palate anomalies have

no significant relationship to the ABH secretor/non-secretor status of the saliva of the subject. Statistical testing by the Chi-square formula showed no significant value. The data obtained revealing the frequency of secretors and non-secretors of the control and experimental subjects tended to agree with the frequency ranges of past research of control groups.



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APPROVAL SHEET

The thesis submitted by Dr. Richard Lee Baker has been read and approved by members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 15, 1970

Date

Charles J. Schumler B.D. D.O.S. M.S.

Signature of Advisor