LACTATE DEHYDROGENASE ISOENZYMES IN SALIVA

by

Ronald Echols

Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Chemistry
Program

Frances W. Smith — 8-22-83
Adviser — Date

Sally M. Hotchkiss — August 23, 1983
Dean of the Graduate School — Date

YOUNGSTOWN STATE UNIVERSITY
August, 1983

ABSTRACT

LACTATE DEHYDROGENASE ISOENZYMES

IN SALIVA

Ronald Echols

Master of Science

Youngstown State University, 1983

A study of lactate dehydrogenase isoenzyme patterns is presented for samples of mixed saliva and parotid fluid. Results of blood serum analysis on the same subjects are included for comparison. A statistical analysis is given for data on samples from normal and "unhealthy" subjects.

The electrophoresis patterns of whole saliva consistently show a progressive increase in concentration from LD_1 to LD_5. However, the isograms of parotid saliva are very similar to those obtained for blood serum, suggesting that an equilibrium exists between the enzymes in these two fluids.

ACKNOWLEDGEMENTS

I extend my profoundest gratitude and sincerest appreciation to my advisor, Dr. Frank W. Smith, for his invaluable direction, incentive, and patience extended to me during this study. I also give special thanks to Dr. Thomas N. Dobbelstein and Dr. Elmer Foldvary for their very constructive review of this research manuscript. I thank Florence Turowski for the translation of a pertinent Polish paper for this research. I also thank Dr. Paul Murphy for carrying out the canalization procedures on the parotid glands. A thank you and acknowledgement is given to Robinson Memorial Hospital for providing many of the subjects used in this research.

I also extend my warmest appreciation to my family for the sacrifices they made during this research.

TABLE OF CONTENTS

PAGE

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION OR SYMBOL	DEFINITION
LD	Lactate Dehydrogenase
M	One of the Lactate Dehydrogenase polypeptide chains
H	One of the Lactate Dehydrogenase polypeptide chains
NAD	Nicotinamide-adenine dinucleotide
NADH	Reduced NAD
ΔA	Symbol for delta-absorbance
μmol	Micromole
mL	Milliliter
R/O MI	Rule out myocardial infarct
C.V.	Coefficient-of-variation
mU/mL	International unit or U/L

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

In recent years there has developed a great interest in the chemistry of saliva and in the use of saliva as an alternative to other body fluids for the diagnosis and monitoring of disease states. This trend can be seen from the number of entries listed in Chemical Abstracts from 1962 to 1982. Table 1 shows this trend.

TABLE 1

NUMBER OF SALIVA ARTICLES FROM 1962-1982

Year	Number of Articles
1962-1966	500
1970	90
1975	90
1980	300
1982	400

The number of articles averaged approximately 100 per year from 1962 to 1975. Thereafter the numbers began to increase greatly to a level of over 400 in 1982.

These investigations have shown the presence of vitamins, lipids, salts, hormones, enzymes, salts, antibodies and proteins. In fact it is now known that the composition of saliva is extremely complex, comparable to that of other body fluids. The following table is an abbreviated listing of the

constituents found in this fluid.[8, 9]

TABLE 2

COMPOSITION OF WHOLE SALIVA

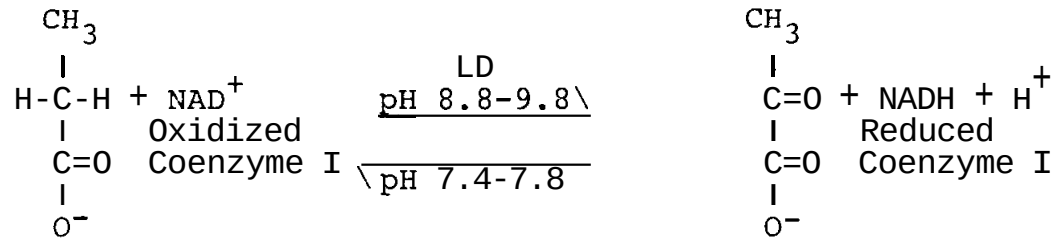
Alkaline phosphatase	Glucose
Acid phosphatase	Insulin
Amylase	Cortisol
Cholinesterase	Immunoglobulina
Lysozyme	Galactose
Esterases	Mannose
Ribonucleases	Fucose
Lacto peroxidase	Total protein
Thrombo plastin	Albumin
Carbon dioxide	Sodium
Urea	Potassium
Uric acid	Chloride
Amino acids	Lactate dehydrogenase
- Proline	Magnesium
- Glycine	Calcium
- Glutamicacid	Inorganic phosphate
- Alanine	Vitamins
- Phenylalanine	Ingested drugs

Lactate Dehydrogenase

The enzyme, lactate dehydrogenase, catalyzes the reaction of L-lactate to pyruvate.[1] This is a reversible reaction with either the oxidation of L-lactate to pyruvate in the presence of the coenzyme NAD^+ being reduced or the reduction of pyruvate to lactate and NADH being oxidized. The following equation describes this reaction.

---

[1]Designated as LD; EC1.l.1.27 by the International Commission on Enzymes.



L-Lactate Pyruvate

The enzyme lactate dehydrogenase is widely distributed throughout the human body. In fact, most tissues in the body contain this enzyme. This fact, in itself, means the measurement of its <u>total</u> enzymatic activity for the diagnosis and treatment of disease processes becomes of less importance when considered alone. Since lactate dehydrogenase is widely distributed throughout the body tissues, <u>total</u> activity does not relate the activity to any specific tissue. However, the lactate dehydrogenase enzyme becomes significant when its "isoenzymes" are studied along with its total enzymatic activity. An isoenzyme can be related to a specific kind of tissue in the body. The <u>isoenzymes</u> are varying molecular forms of the same enzyme. They catalyze the same chemical reaction but differ in certain biochemical, physical, and immunological properties.[2,3]

In the case of lactate dehydrogenase, its isoenzymes are distributed throughout the body in much more predictable patterns than total LD. Lactate dehydrogenase is a tetramer consisting of four polypeptide chains which are designated <u>H</u> or <u>M</u>. By considering different combinations of these <u>H</u> and <u>M</u> polypeptide chains, five different isoenzymes

of lactate dehydrogenase can be formed.

TABLE 3

THE ISOENZYMES OF LACTATE DEHYDROGENASE

Peptide Chain Combination	Isoenzyme
HHHH(H_4)	LD_1
HHHM(H_3M)	LD_2
HHMM(H_2M_2)	LD_3
HMMM(HM_3)	LD_4
MMMM(M_4)	LD_5

When lactate dehydrogenase enzyme is subjected to electrophoresis, it separates into the above five fractions (LD_1 to LD_5). In this research, the samples were placed at a cathode application point. The LD_1 fraction was the fastest moving (the most anodic) and LD_5 the slowest. The "H" chain is named such because of its isolation from cardiac tissue. The "M" chain is so named because it was isolated from skeletal muscle. This enzyme is actually an intracellularenzyme. It enters the blood after either normal breakdown of tissue and cells or injury to tissue due to disease.

The lactate dehydrogenase isoenzyme patterns of blood serum samples are well documented both in normal individuals and different disease states.[2] For instance, the characteristic pattern observed after a myocardial infarct (heart attack) is used to help diagnose this disease.[14] With the increasing interest in saliva for clinical testing,[4, 5, 7]

it seemed appropriateto investigate the **LD** isoenzyme patterns of this body fluid. A search of the literature revealed no comprehensive study of **LD** isoenzymes in saliva of humans depicting differences in healthy and disease states. However, after completion of this research project, I became aware of a single report in the literature describing the measurement of saliva LD patterns.[7] This study by Kubicz, et al. did not deal with any clinical significance of **LD** in saliva. The study and conclusions will be summarized and compared with this research later.

Salivary Glands

The salivary glands are a group of glands located in the oral cavity and together they produce "whole" or mixed salivary fluid. The major salivary glands are paired in nature. They are the parotid, submandibular, and the sublingual.

The largest of the salivary glands is the parotid. The paired glands are located in the upper near posterior position on either side of the oral cavity. These irregularly shaped glands lie beneath the skin and secrete saliva through the "Stensen's ducts."

The second pair of major salivary glands is the submandibular glands. These glands are located at the bottom of the oral cavity. These glands secrete through the "ducts of Wharton." The submandibular glands are the second largest of the salivary glands.

The third largest of the salivary glands are the sublingual. They are located in the floor of the oral cavity and they secrete saliva via the "ducts of Bartholin."

The other salivary glands are considered minor in their contribution to whole saliva. Their location beneath the epithelium is not characterized by any one position, but are located in most parts of the oral cavity.[8, 9]

Saliva

The roles of salivary fluid are also complex in nature. They can be summarized under six headings. Firstly, saliva acts as an antimicrobial agent in the oral cavity. This action is carried out by lysozyme and IgA. The lysozyme has the ability to break down the bacterial cell walls of microorganisms not normally found in the oral cavity. This muramidase enzyme (lysozyme) breaks down the cell walls of bacteria that have muramic acid. The IgA is an immunoglobulin (part of the complex auto-immune system of the body) that counteracts the action of microorganisms by being an antibody to these antigens. It reacts with the bacteria by forming complexes, thus rendering the bacteria harmless.

Secondly, saliva serves to lubricate the oral cavity which facilitates speaking and swallowing. The saliva also provides a protective substance for the mucous membranes in the oral cavity.

Third, there is a cleansing role that saliva serves. It mechanically removes debris such as food, bacteria, and celluar material from the oral cavity.

Fourth, saliva acts as a buffering agent by means of its contents of bicarbonate, phosphate, and amphoteric proteins.

Fifth, saliva functions in the maintenance of the teeth. It provides minerals to help maintain the integrity of the teeth and postruptive maturation. Calcium and phosphate from saliva help to neutralize plaque and thus prevent tooth decay. Saliva also forms a film over the teeth which helps protect them from abrasion.

Lastly, saliva has a function in the digestion of food. The saliva contains the enzyme amylase which acts on starches. Also saliva serves to lubricate the food to make its movement easier during swallowing.[8, 9, 10]

In any study of salivary constituents, the flow rate and other factors discussed below must be taken into account in any interpretation of the data obtained.

It has been shown that the concentration of some constituents in saliva is affected by the flow rate. This flow rate is not under the influence of the parasympathetic nervous system. The unstimulated flow rate of whole saliva is approximately 0.008 mL-1.85 mL/minute for healthy individuals. This unstimulated flow rate varies during the day. The rate during sleep is practically zero. There are also seasonal variations and differences due to posture, with the fastest rate in the standing position.

The composition is also affected when the salivary glands are stimulated. With respect to the gustatory

stimulation of the taste buds, the flow rate is seen to increase with increase in the intensity of the stimulation. However, the increase in flow rate reaches a maximum value and no further increase in the stimulus will produce any further increase in the flow rate of saliva. This maximum value is characteristic for each individual. The strongest and most effective gustatory stimulation is caused by acids such as citric acid. Stimulation can also be accomplished by chewing on inert materials (e.g. parafilm). This mechanical stimulation is less effective than the gustatory stimulation.

Other factors causing variations in flow rate and concentration of salivary constituents are: the age of individuals (lesser flow rate with age), sex (this is possibly due to the size of the glands themselves), drugs (some drugs suppress flow rates and some stimulate by influence of reflex action and the central nervous system), source of saliva (the amount of a specific constituent may vary due to the salivary gland producing it, e.g., blood group antigens are found in much higher concentrations in the lip mucous gland secretions than in the submandibular saliva), diet (due to a reflex effect on salivary flow rate), and plasma levels (the concentration of constituents in the plasma has some effect on the concentration in the saliva, although the ratio between the two fluids remains the same).[4, 8, 9, 10]

Therapeutic drug monitoring is another area of intense interest in the study of saliva for a number of

reasons. First, salivary testing can be carried out without invasive collection techniques such as used in the study of plasma (venipuncture). Also, it seems that in some instances the concentration of the therapeutic drugs in saliva, after stimulation, is related to the concentration in plasma. A good review of recent developments is given by M. Danhof and D. D. Breimer in the article "Therapeutic Drug Monitoring in Saliva."[5] They point to studies which indicate that the concentrations of some drugs in saliva are the same as the protein unbound concentration in plasma. The plasma "total" concentration represents both the bound and unbound drugs. Some clinicians emphasize the importance of unbound levels as being the physiological active components. The drugs may enter the saliva by a passive diffusion process or are actively transported into the saliva.

Danhof and Breimer emphasize the advantages in having a stimulated saliva sample for the study of drug constituents, for example:

1. One can obtain large volumes much more easily.
2. It helps to maintain the narrow band of pH of approximately 7 (important in the measurement of weak acidic and basic drug compounds).
3. There is less variability between the saliva of subjects.

A disadvantage of stimulating with some materials that subjects chew, such as parafilm, is that there can be absorption of some drugs onto the parafilm. This absorption will then cause erroneously low results when the sample is analyzed.

Danhof and Breimer state that a most essential prerequisite to have the saliva "therapeutic drug monitoring" be significant, is the establishment of a correlation between drug concentrations in plasma and saliva over a wide concentration range. To show this correlation, they reported the established S/P ratios (saliva to plasma) of a wide range of drugs, including theophylline, lithium, phenobarbitol, phenytoin, primidone, ethosuximide, carbamazepine, digoxin, and procainamide.

They concluded that there seems to be a better correlation between saliva concentrations and the pharmacological effect of the drugs than for plasma and pharmacological response.

Purpose of This Investigation

The goal of this research project was to determine the total activity and distribution of LD isoenzymes in whole saliva and in parotid fluid and to compare these with serum values. A further goal was to attempt to establish "normal" ranges for "healthy" individuals as well as to explore any possible relationship between the salivary LD isoenzymes values with specific disease states.

CHAPTER 2

METHODS, MATERIALS, AND EQUIPMENT

Mixed (Whole) Saliva

Patients were first given parafilm® to chew for three to five minutes. Then the patients were instructed to collect the "whole" saliva in clean plastic containers. The appearance of the samples before centrifugation was cloudy and generally a light greyish color.

The mixed (whole) saliva contains contributions from all salivary glands (parotid, submaxillary, sublingual, and minor gland secretions), shed mammalian and bacterial cells, leukocytes, and other particulate matter found in the oral cavity. The mixed saliva may also contain cantamination from material coughed up from the lower respiratory tract, coughed-up minute flecks of material in the mouth, material coming from the nasopharynx, nasal cavity, and paranasal sinuses. Even material regurgitated may contaminate the saliva with gastric or esophageal contents. Additional contamination may occur by outside food, drink, inhaled tobacco smoke, and chewing other materials.[9]

An analysis of the mixed saliva without any prior preparatory treatment of the samples was originally attempted. This produced a number of problems which suggested that pretreatment by centrifugation was necessary to remove any

particulate matter. The supernatant was then removed from the sediment and analyzed or the samples were kept at room temperature overnight until testing. This temperature was chosen because cold temperatures are known to affect lactate dehydrogenase isoenzyme electrophoretic patterns.[1, 2]

The problems associated with analyzing saliva samples without centrifugation were: 1) inconsistent results of the "total" LD enzymatic activity as analyzed by spectrophotometry (interference with linear kinetic rate absorbance changes at 340 nm by the particulate material), 2) production of electrophoretic isoenzyme patterns that were very non-uniform and of poor quality, 3) and isoenzyme patterns that were inconsistent in fraction concentrations which were unable to be scanned readily because of the heavy background noise.

Parotid Fluid

Samples were also collected from the parotid gland to compare its patterns with that of mixed (whole) saliva. Attempts were first made to collect parotid saliva samples using a modified Carlsen-Crittenden apparatus. This collector was developed in 1916 by Lashley.[8, 9] The main advantage of the collector is that parotid fluid can be collected without the problem of contaminants from the oral cavity. The collector has a design of two concentric circles with metal stems from the outside to holes inside the circles. The inside circle is designed to fit over the parotid duct

opening (Stensen's duct). The other (outer) concentric circle's stem is attached to a rubber bulb. The collector is held in place with the fingers and the bulb is used to expel the air from the outer circle and draws the area surrounding the parotid opening into the outer circle. The saliva or parotid fluid is then free to flow into the circle and through the hole and out the metal stem into the collecting tube. The dimensions of the inner chamber are 10 mm diameter and the outer 20 mm diameter. The collector has a depth of 4 mm. I experienced two main disadvantages with the use of the collector. Some patients had difficulty maintaining the collector tight without movement if they were sucking or chewing on a substance to stimulate saliva flow. Secondly, too much air-suction can be applied so that the buccal mucosa is sucked into the inner circle and occludes the opening.

In a telephone conversation with Dr. Irwin D. Mandel (of the School of Dental and Oral Surgery and Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York), he suggested the following helpful hints in using the parotid collector:

1. Make sure the inner chamber is not occluded by the duct opening or the area around the opening.
2. A light can be used to help locate the parotid duct opening.
3. Citric acid can be put on the tongue and one can see the place where the fluid comes out.
4. One suction with a rubber bulb should hold the apparatus in place approximately 10-15 minutes.

5. The rate of collection unstimulated should be about .03 ml/minute and the stimulated rate about 0.5 ml/ minute.

6. If the apparatus fails, parotid fluid can be collected by taking a gauze roll used by dentists and placing it at the top of the oral cavity around the parotid duct opening 5-10 minutes. The gauze is then removed and the fluid squeezed out into a container.

With the help of an oral surgeon (Dr. Paul Murphy of Youngstown, Ohio) "pure" parotid fluid samples were obtained on two occasions by use of a sterile Rabinov Sialography Set. This catheter had a metal needle at one end which was attached to an approximately one foot piece of plastic tubing. At the other end of the plastic tubing it had a luer loc to which a syringe could be attached if desired. The metal needle was removed from the canalization set because it was directional and we wanted fluid flowing out. Firstly, he injected a small amount of novocain to numb the area where the tubing was to be inserted. The catheter tubing was inserted into the parotid duct and he applied two stitches to hold the catheter in place. The luer loc end of the catheter was held in a test tube to catch the parotid fluid. It took about one-half hour to collect 6 ml. of fluid. Parotid samples were collected on two occasions.

Blood Serum

Blood samples were obtained from the same patients who provided saliva just before or right after the saliva collections. The blood samples were collected in SST® tubes (a serum separator tube) and allowed to clot at room

temperature and then centrifuged to obtain the serum. For reasons mentioned above, the serum specimens were stored at room temperature until assayed. The serum specimens were assayed for total enzyme activity and electrophoretic patterns obtained by the same method as described above for saliva samples.

Equipment

The total enzymatic activity in the serum and saliva was assayed by means of the Gilford System 3500®, which is a computer directed analyzer. The cornerstone of the System 3500 is a reference spectrophotometer providing built-in electronic temperature control at 30°C (temperature at which the total LD's were run). The system has a 56-sample transport which contains 14 racks capable of holding the sample cups and reaction strips. The instrument is programmed to perform enzyme assays via magnetic program cards that direct the instrument's computer to guide the operator through the procedure by printing guidelines for setup of each enzyme assay. The bottle of reagent substrate for LD determinations is placed on a tower which is capable of being adjusted to the desired volume to be dispensed. The sample pickup volume can also be varied. The sample size for LD assays in this study was 50 microliters. The reagent volume was 0.7 ml.

The total LD concentration activities were obtained by using a LD substate reagent of pyruvate, tris buffer, NAD,

and L-lactate to which the samples containing LD enzyme were added. The spectrophotometer on the Gilford 3500® follows the absorbance changes as NAD is reduced to NADH during the reaction of L-lactate topyruvate. The calculation presented in Fundamentals of Clinical chemistry[1] for the LD activity in International Units at 30°C is as follows:

$$mU/ml = \frac{\Delta A}{min} \times \frac{1000}{6.22} \times \frac{3.1}{0.1} = 4985 \times \frac{\Delta A}{minute}$$

where 3.1 = total volume in cuvet, in ml

0.1 = volume of serum specimen, in ml

6.22 = millimolar absorbtivity of NADH at 340 nm (unit = ml x $mmol^{-1}$ x cm^{-1})

1000 = converts mmol to μmol

$\frac{\Delta A}{min}$ = average absorbance change (decrease) per min

A Sero-fuge® centrifuge from Clay-Adams -0511 was used to obtain supernatant from the saliva samples. It has a fixed speed of 3400 rpm's. Mixed (whole) saliva samples were spun for five minutes and the supernatant used in the assay of "total" LD activity and the electrophoresis separations.

Helena Electrophoretic Equipment was used for the electrophoresis portion of this research. The LD samples (both blood and saliva) were electrophoresed or separated into their individual fractions (isoenzymes) on cellulose acetate medium. The electrophoresis was for ten minutes after which the plates were removed from the electrophoretic chamber and sandwiched onto other cellulose acetate plates

containing lactic dehydrogenase enzyme substrate. The sandwiched plates were then placed onto a 37°C heating block between metal weights for twenty-five minutes to develop the separated isoenzyme patterns. At the end of the twenty-five minutes, the sandwiched plates were then separated and allowed to dry.

A scanner and densitometer from Helena Laboratories@ was used to scan the lactate dehydrogenase separated fractions. The Quick Quant II® attached to the Auto Scanner Flur-VIS® provided high speed, automatic quantitation and printout of data obtained by the densitometer. Both the percentage of the total and the concentration of the fraction were computed for the isoenzymes.

CHAPTER 3

RESULTS

Whole Saliva and Blood Serum LD Electrophoresis

In the case of blood serum, the patterns from "normal" individuals always showed the first three fractions with the greatest concentration of the five. Only in certain disease states did this general pattern alter. Figure 1 is an illustration of this "normal" lactate dehydrogenase pattern.

The fractions from left to right are: $LD_1(HHHH-H_4)$, $LD_2(HHHM-H_3M)$, $LD_3(HHMM-H_2M_2)$, $LD_4(HMMM-HM_3)$, $LD_5(MMMM-M_4)$. The patterns were obtained on chart paper by scanning the LDH fractions on the cellulose acetate strips from the electrophoresis. A cathode application of the samples was made and the LD fractions migrated in the electric field toward the anode according to their mobilities. The LD_1 was always the fastest fraction followed by LD_2 to LD_5. Figure 2 below shows (an artist's) drawing of the bands of LD isoenzymes fractions as they appear to the eye on cellulose acetate plates under fluorescence.

When the mixed saliva samples were run, the patterns showed a very interesting composition. All the samples showed a "reversed" configuration from that of blood serum, that is, the LD_1 fraction was always the lowest concentration

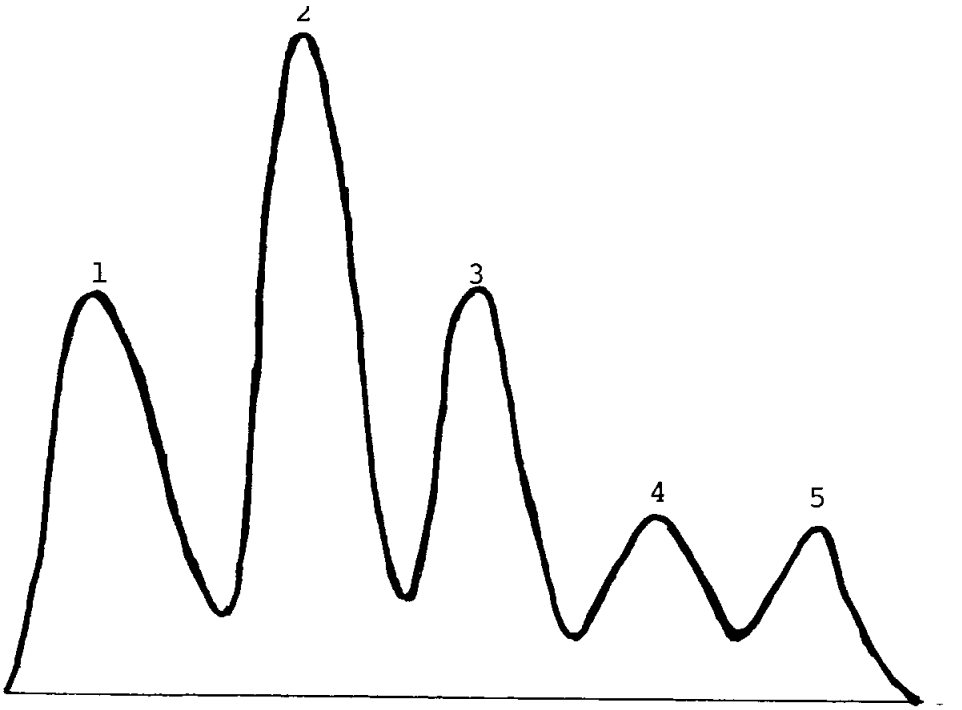


Fig. 1. Normal Blood Serum LD Isoenzyme Pattern

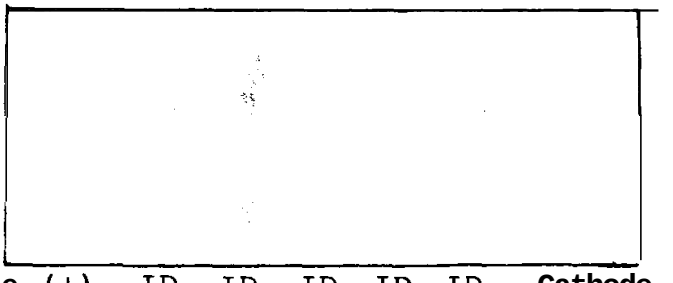


Fig. 2. Cellulose Acetate Separation of LD Showing the Five Bands.

of the five and LD_5 always the greatest concentration. This progression in concentration from LD_1 to LD_5 held true in over 100 different samples analyzed (see Figure 3).

The range of disease states included in this study is shown in Table 4.

TABLE 4

SUMMARY OF TYPES OF INDIVIDUALS USED IN SALIVARY STUDY

Myocardial infarction	Colon mass
Rule out myocardial infarction	Coronary insufficiency
Automobile accident	CA metastasis of lung
Gallbladder disease	Skull concussion
Hepatitis	Collagen's disease
Hernia and esophagus infection	Hemolytic anemia
Congestion heart failure	Cirrhosis of liver
Emphysema	Hypertension
Diabetes	Right parotidectomy
	Cystic fibrosis
	"Normal" population

The normal samples in this research came from a large number of "apparently healthy" individuals characterized by not being hospitalized or aware of any illness at the time of sample collections. The ages of the subjects was from five years to eighty-three years. The saliva and blood Samples (collected as previously described) were analyzed for the <u>total</u> lactate dehydrogenase enzymatic activity. The results of the analysis are given in Table 5.

<u>Comparison Between Saliva and Blood Results</u>

A comparison between the total lactate dehydrogenase enzyme activities of the blood and saliva from the same

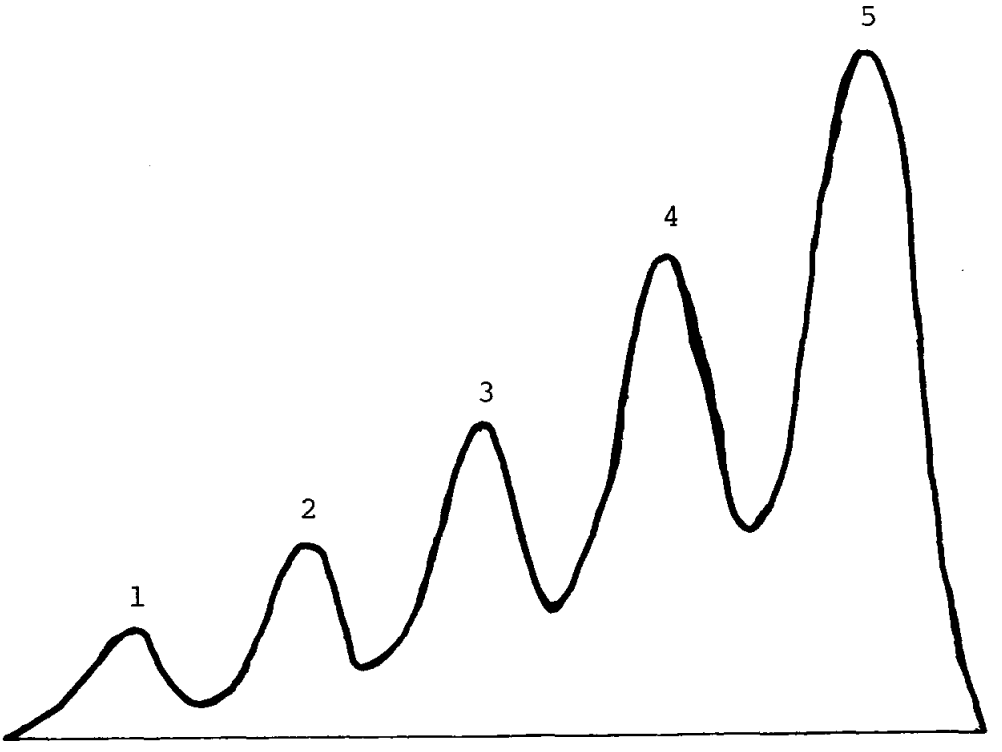


Fig. 3. General Mixed Saliva Pattern Produced by Electrophoresis Showing the Peak Height Relationships.

TABLE 5

TOTAL LD ACTIVITY OF SALIVA AND BLOOD IN (mU/ml)

Column 1		Column 2		Column 3	
Saliva	Blood	Saliva	Blood	Saliva	Blood
81	178	297	878	512	269
96	262	276	146	515	137
65	111	285	500	619	380
84	145	274	344	612	500
134	365	295	337	681	858
152	150	271	142	612	271
186	149	249	125	870	185
173	152	228	150	846	254
180	449	269	150	947	165
134	312	210	107	999	335
185	185	261	132	1020	272
153	124	353	172	1150	--
158	105	315	188	1789	177
102	137	332	130	1378	144
172	111	371	188	1630	211
210	144	441	165	173	--
272	314	416	445	97	--
262	177	455	152	138	--

subjects showed no apparent correlation. The occasional elevation of blood serum levels could, in many cases, be traced to a known disease process, but the variations in concentrations of the total LDH in salivary samples could be a function of the rate of secretion, the differences in individual stimulation of salivation, and the variations of individuals' salivary glands to form and produce saliva, rather than the function of some associated disease.

Since the total enzymatic concentration of LD in saliva revealed little that was conclusive, the next step was to review the relative proportion of the individual

isoenzyme fractions as percentages of the total activity. Table 5 shows the results for 11 "healthy" individuals and 40 people who were known to be suffering from a specific health problem. Although the total activities varied from 81 to 1750 mU/ml, the percentages of the five fractions always adds up to 100%. Any possible relationship between the various disease states with a change in one or more of the isoenzyme fractions' percentages was investigated. Figures 4 through 8 are histograms showing the distribution of each LD fraction in the samples studied.

The LD fraction 1 for saliva formed a uniform histogram typical of a "gaussian" distribution. There was one outlying value with this study group. This "outlier" corresponded to a hospitalized patient having myocardial infarct ruled out as a diagnosis. The median percentage concentration of LD fraction 1 was between 4-5% (Figure 4).

LD fraction 2 showed a similar graphic as that of fraction 1. It showed a semblance of a gaussian distribution. There were two "outliers" within this fraction. The diagnoses of these outliers were "chest pain" and "thrombus arthistis." The median percentage concentration of LD fraction 2 fell between 7 and 8% (Figure 5).

The third fraction had a wide concentration spread from 8% to 26%. The median percentage concentration of LD fraction 3 was 15.5%. There was one outlier of 6.3%. The diagnosis for this outlier was chest pain.

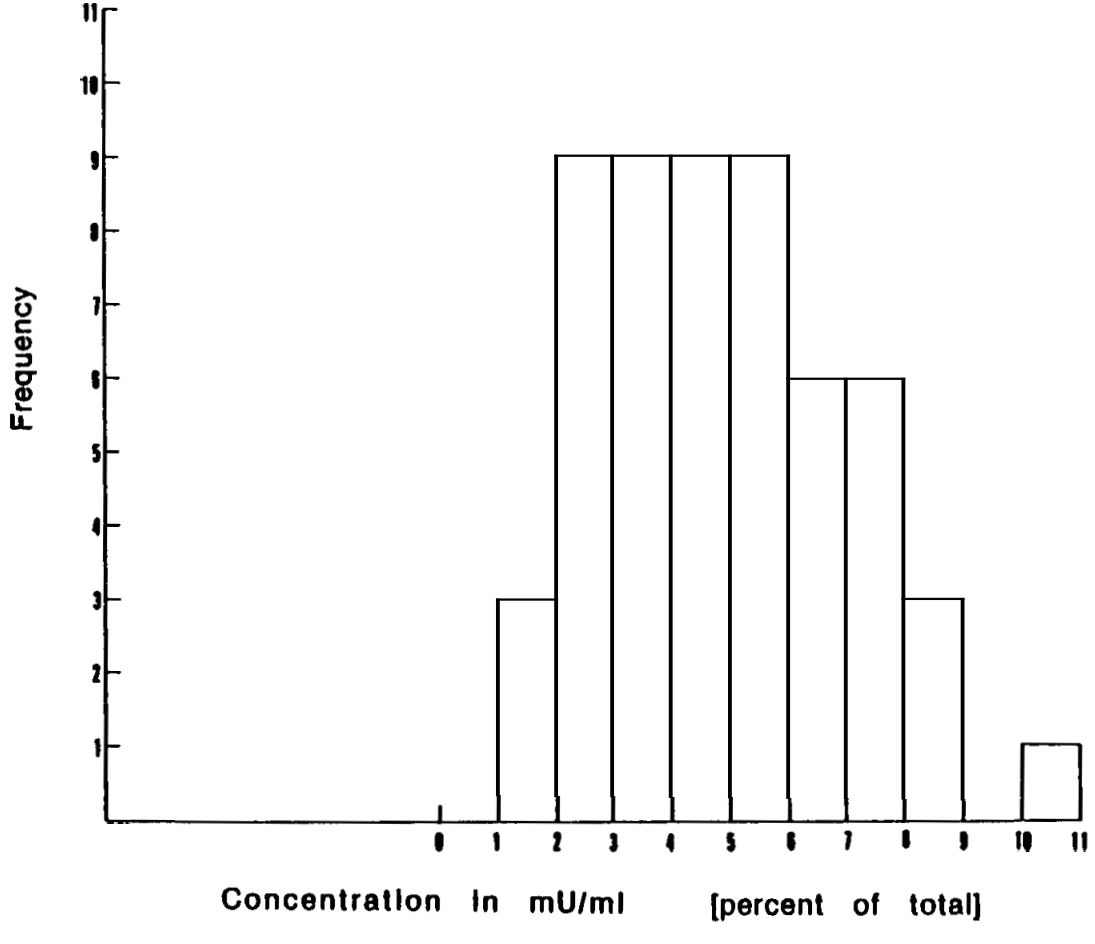


Fig. 4. LDH FRACTION 1 [Saliva] Showing its histographic pattern of the subjects in the study.

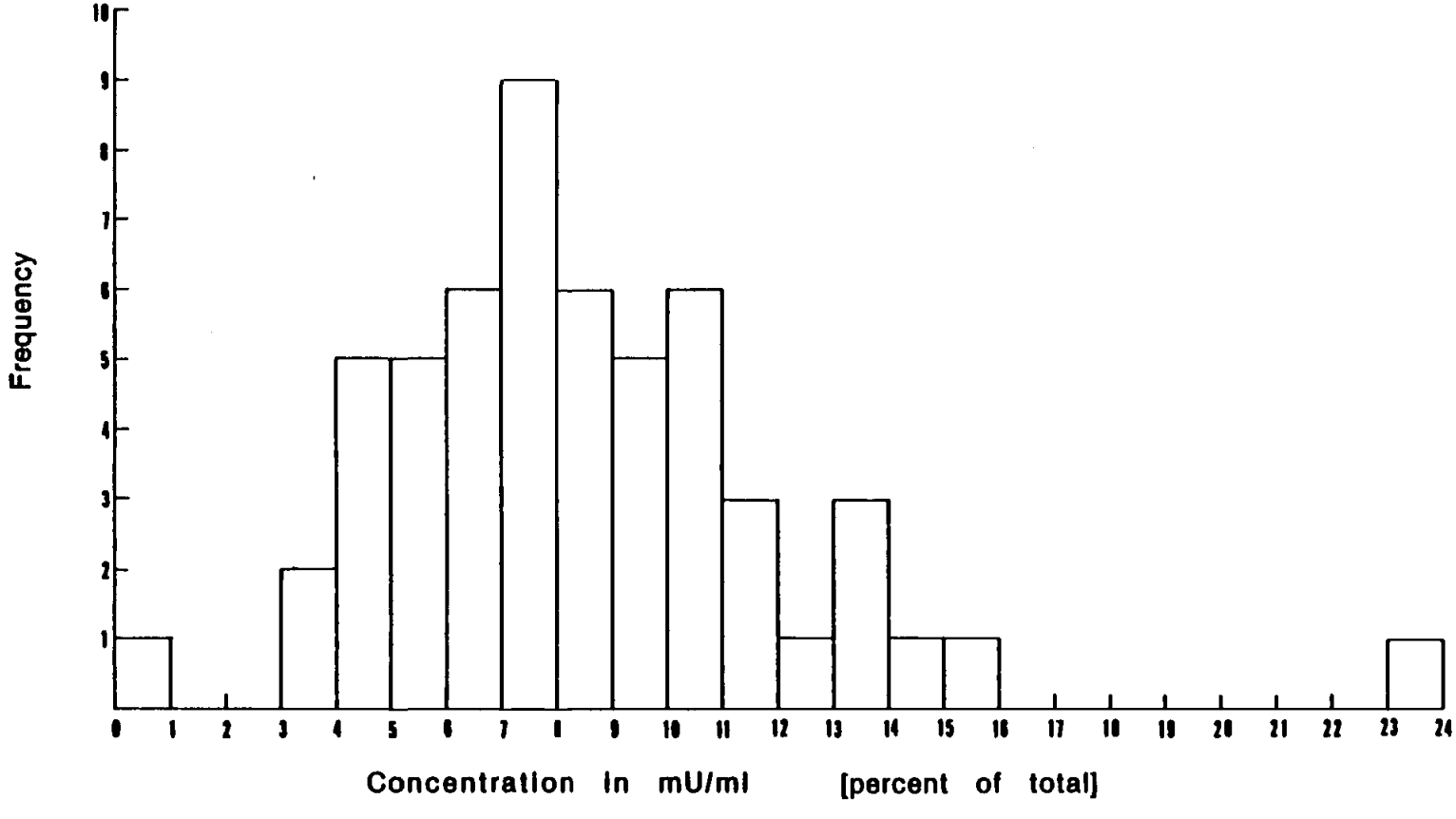


Fig. 5. LDH FRACTION 2 [Saliva] Showing its histographic pattern of the subjects in the study.

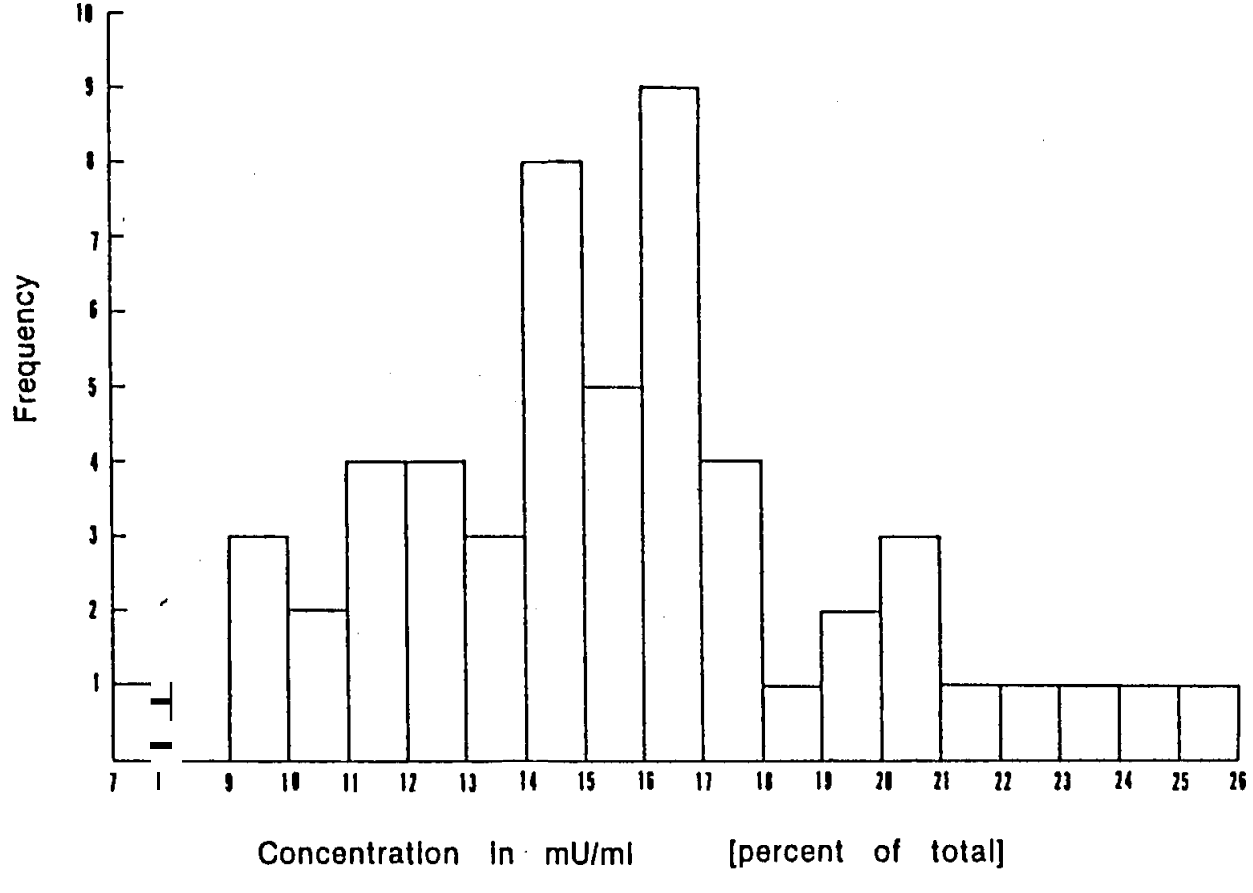


Fig. 6. LDH FRACTION 3 [Saliva] Showing its histographic pattern of the subjects in the study.

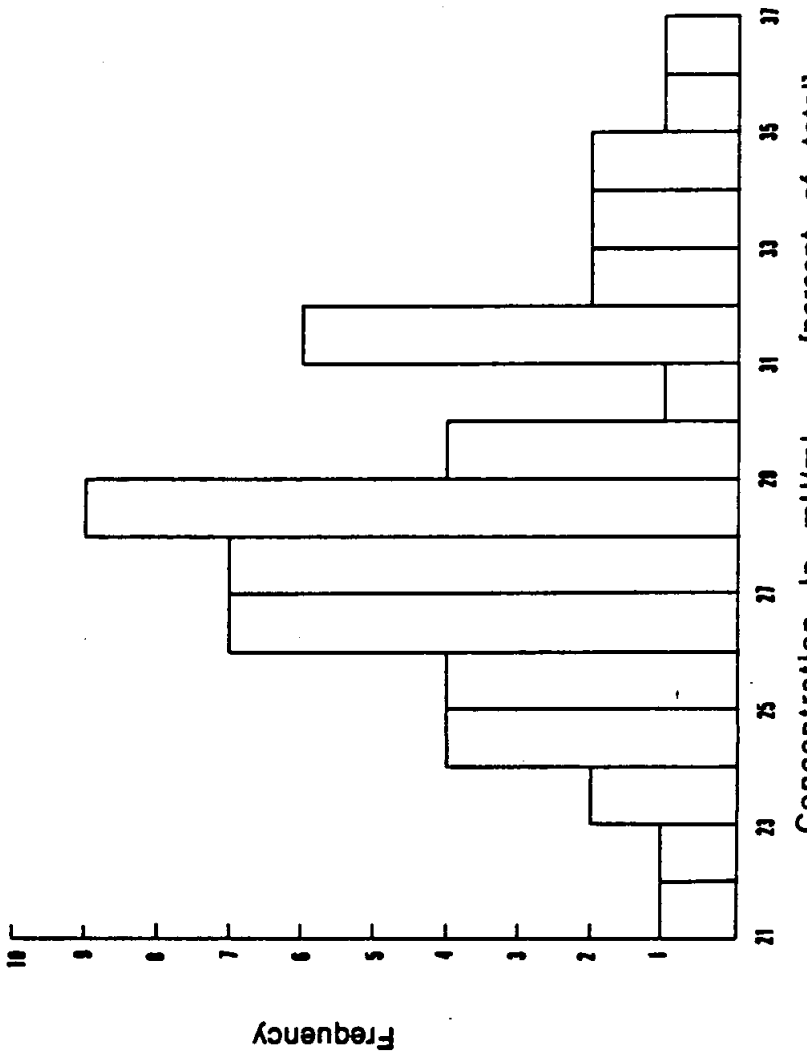


Fig. 7. LDH FRACTION 4 [Saliva] Showing its histographic pattern of the subjects in the study.

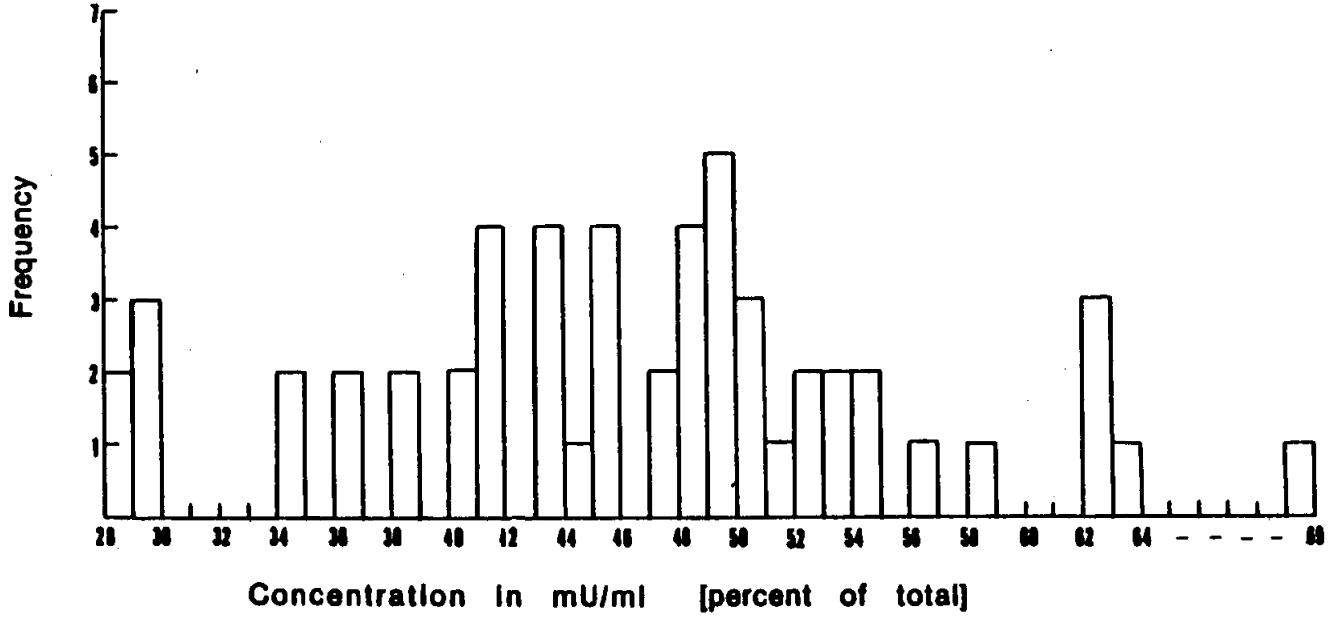


Fig. 8. LDH FRACTION 5 [Saliva] Showing its histographic pattern of the subjects in the study.

The fourth saliva **LD** fraction also took the shape of a gaussian distribution. The median percentage of LD_4 was 28%. There were no significant outliers with this fraction. The distribution spread from 21% to 36%.

The distribution for fraction five was less uniform than the other four fractions. The median percentage concentration was 46%. There were gaps between the columns of frequencies. This fraction was characterized by two clusters of outliers. They were about equal distance on each side of the main distribution. The individual subjects who made up these clusters did not appear to have any particular relationship to each other by diagnosis. The diagnoses of the cluster on the left were: cystic fibrosis, CA metastasis of lung, diabetes, and rule out ischemic attack and dehydration. The diagnoses of the cluster on the right were: cellulitis lower extremity, drug induced hemolytic anemia, diabetes, and apparently normal. The one significant outlier with the fifth fraction was "chest pain."

Relationship Between **LD** Fractions of Saliva and Serum

As mentioned previously, it quickly became apparent in this study that any direct comparison of the total enzymatic activity concentrations of the subjects' serum and saliva had little significance. In order to compare the percentages of the individual fractions of saliva with those of serum, ratios were calculated for each fraction. The results are summarized in Table 6 and Figures 9 through 13.

TABLE 6

THE LISTING OF SUBJECTS WITH DEMOGRAPHIC INFORMATION, DIAGNOSIS, WITH TOTAL ENZYME ACTIVITIES IN mU/ml AND LD FRACTIONS IN PERCENTAGES

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
1(a).	M	60	R/O MI[a]	Saliva	81.0	5.4	6.0	10.5	27.6	50.5
				Serum	178.0	27.2	33.4	20.2	9.0	10.1
				Ratio	0.5	0.2	0.2	0.5	3.1	5.0
(b).				Saliva	441.0	2.6	5.9	14.0	3.0	47.5
				Serum	165.0	26.6	57.2	19.7	8.6	7.9
				Ratio	2.7	0.1	0.1	0.7	3.5	6.0
2.	M	19	Auto Accident	Saliva	947.0	2.1	7.3	18.7	32.9	39.0
				Serum	165.0	25.2	31.4	19.3	10.9	13.1
				Ratio	5.7	0.1	0.2	1.0	3.0	3.0
3.	F	50	Gallbladder	Saliva	1020.0	5.0	10.0	18.6	30.8	35.6
				Serum	272.0	10.3	12.1	21.2	31.1	25.3
				Ratio	3.8	0.5.	0.8	0.9	1.0	1.4
4.	M	50	Poss. Hepatitis	Saliva	619.0	3.4	11.3	21.1	27.9	36.4
				Serum	380.0	17.1	22.7	14.3	10.7	35.2
				Ratio	1.6	0.2	0.5	1.5	2.6	1.0

[a]R/O MI = Rule out myocardial infarct

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
5.	F	69	Hernia	Saliva	353.0	4.6	9.9	20.1	31.3	34.2
				Serum	172.0	16.6	14.8	16.4	16.0	36.2
				Ratio	2.1	0.3	0.7	1.2	2.0	0.9
6.	F	16	Hepatitis	Saliva	612.0	3.1	7.6	15.9	34.1	39.4
				Serum	500.0	18.3	21.5	14.5	11.2	34.5
				Ratio	1.2	0.2	0.4	1.1	3.0	1.1
7.	M	48	MI	Saliva	210.0	7.4	9.7	15.4	27.0	40.6
				Serum	--	--	--	--	--	--
				Ratio	--	--	--	--	--	--
8.	M	59	-Congestive heart -Emphysia -Diabetes	Saliva	272.0	5.1	3.9	11.5	29.7	49.7
				Serum	314.0	23.4	27.4	18.1	9.9	21.3
				Ratio	0.9	0.2	0.1	0.6	3.0	2.3
9.	M	87	-Mass of sigmo colon -Abdominal pain	Saliva	262.0	5.6	8.4	17.1	27.5	41.4
				Serum	177.0	30.2	32.9	21.3	9.4	6.1
				Ratio	1.5	0.2	0.3	0.8	2.9	6.8
10.	F	60	Coronary insufficiency	Saliva	297.0	6.8	8.3	14.4	25.0	45.5
				Serum	878.0	42.8	36.5	13.6	4.5	2.7
				Ratio	.3	.3	.2	1.1	5.6	16.9

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
11.	F	65	CA(lung)	Saliva	1000.0	6.3	13.6	24.4	27.2	28.2
				Serum	335.0	17.5	31.5	23.1	14.1	13.9
				Ratio	3.0	0.4	0.4	1.1	1.9	2.0
12.	M	48	Collulitis lower extrem- ity	Saliva	134.0	2.9	5.1	11.6	20.7	59.8
				Serum	365.0	30.4	34.4	20.6	8.6	6.0
				Ratio	0.4	0.1	0.2	0.6	2.4	10.0
13.	M	19	Skull concussion	Saliva	416.0	5.8	9.3	15.6	26.1	43.1
				Serum	445.0	21.7	23.4	22.5	16.0	16.4
				Ratio	0.9	0.3	0.4	0.7	1.6	2.6
14.	F	-	Chest pain	Saliva	276.0	1.2	0.2	6.5	23.0	69.1
				Serum	146.0	27.8	33.6	19.5	10.0	9.1
				Ratio	1.9	0.1	0.1	0.3	2.3	7.6
15.	F		Thrombus Arthritis	Saliva	512.0	1.7	24.1	11.5	25.0	37.7
				Serum	16.4	25.9	25.0	16.6		
				Ratio						
16.	F	-	Congestive heart failure	Saliva	285.0	4.5	6.5	14.0	28.2	46.7
				Serum	500.0	19.4	19.9	12.5	11.6	36.6
				Ratio	0.6	0.1	0.3	0.3	2.4	1.3

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
17(a).	F	25	Collagen's	Saliva	315.0	2.9	6.4	14.7	27.6	48.3
				Serum	188.0	27.0	38.1	21.5	7.6	5.8
				Ratio	1.7	0.1	0.2	0.7	3.6	8.3
17(b).	F	25	Collagen's	Saliva	152.0	6.8	4.2	9.2	24.2	55.7
				Serum	150.0	29.9	35.9	18.1	8.2	7.9
				Ratio	1.0	0.2	0.1	0.5	3.0	7.1
18.	M	49	Drug induced hemolytic anemia	Saliva	274.0	3.7	3.5	9.4	23.5	59.9
				Serum	344.0	35.4	30.7	15.5	8.0	10.3
				Ratio	0.8	0.1	0.1	0.6	2.9	5.8
19(a).	M	71	R/O MI	Saliva	332.0	7.0	12.3	18.0	24.3	38.4
				Serum	130.0	24.0	29.5	18.3	11.3	16.5
				Ratio	2.6	0.3	0.4	1.0	2.1	2.3
19(b).	M	71	R/O MI	Saliva	186.0	10.5	13.2	17.3	23.6	35.4
				Serum	149.0	27.4	34.1	19.7	10.4	8.3
				Ratio	1.3	0.4	0.4	0.9	2.3	4.3
20.	F	42	Diabetes	Saliva	173.0	4.1	4.1	9.2	21.6	61.0
				Serum	152.0	26.8	33.0	20.3	7.7	12.3
				Ratio	1.1	0.2	0.1	0.5	2.8	5.0

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
21.	F	83	Gallbladder	Saliva	1789.0	2.2	10.3	23.1	27.9	36.4
				Serum	177.0	26.1	33.7	19.8	9.6	10.7
				Ratio	10.1	0.1	0.3	1.2	2.9	3.4
22.	M	31	Diabetic	Saliva	1378.0	7.2	14.9	22.1	26.0	29.8
				Serum	144.0	27.6	30.1	16.0	10.4	16.0
				Ratio		0.3	0.5	1.4	2.5	1.9
23.	F	--	Chest pain	Saliva	870.0	2.1	6.7	13.7	32.0	45.5
				Serum	185.0	21.8	31.8	23.1	11.1	12.3
				Ratio	4.7	0.1	0.2	0.6	2.9	3.7
24.	F	--	Ascites	Saliva	295.0	8.4	7.2	15.2	28.2	41.0
				Serum	337.0	22.3	39.3	21.7	7.6	9.1
				Ratio	0.9	0.4	0.2	0.7	3.7	4.5
25.	M	--	Hypertension	Saliva	846.0	4.9	9.4	16.3	26.8	42.6
			Diabetes	Serum	254.0	26.0	31.9	23.8	9.5	8.8
			Heart disease	Ratio	3.3	0.2	0.3	0.7	2.8	4.8
26(a).	F	56	Right	Saliva	271.0	6.0	8.7	16.3	25.9	43.2
			Parotidectomy	Serum	142.0	25.4	35.5	22.4	9.6	7.2
			(Before)	Ratio	2.0	0.2	0.3	0.7	2.7	6.0

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
26(b).	F	56	Right Parotidectomy (After)	Saliva	330.0	3.0	10.8	20.0	26.8	39.4
				Serum	142.0	25.4	35.5	22.4	9.6	7.2
				Ratio	0.8	0.1	0.3	0.9	2.8	5.4
27.	F	25	Hypothyroid-ism	Saliva	65.0	3.0	7.5	15.0	28.0	46.0
				Serum	111.0	23.6	30.6	21.9	12.5	11.5
				Ratio	0.6	0.1	0.2	0.5	1.4	2.6
28.	F	16	Hepatitis	Saliva	612.0	3.1	7.6	15.9	34.1	39.4
				Serum	271.0	24.4	23.5	15.0	9.0	27.5
				Ratio	2.3	0.1	0.3	1.1	3.6	1.4
29.	F	79	R/O MI Poss. CVA	Saliva	1630.0	2.9	13.0	24.5	30.7	28.8
				Serum	211.0	34.1	33.3	17.6	7.4	7.6
				Ratio	7.7	0.9	0.4	1.4	4.2	3.8
30.	M	63	Cirrhosis	Saliva	180.0	1.5	3.3	15.3	29.2	50.6
				Serum	449.0	12.5	35.7	19.2	6.3	11.2
				Ratio	0.4	0.1	0.1	0.8	4.6	4.5
31.	M	50	Pulmonary Emphysema	Saliva	371.0	2.0	10.7	19.9	31.0	36.4
				Serum	188.0	25.7	35.2	21.7	8.5	8.9
				Ratio	2.0	0.1	0.0	0.9	3.6	4.1

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
32(a).	F	20's	KI Toxicity	Saliva	134.0	3.0	6.7	15.7	28.0	46.6
				Serum	312.0	12.1	19.7	14.2	13.9	40.2
				Ratio	0.4	0.3	0.3	1.1	2.0	1.2
32(b).	F	20's	KI Toxicity	Saliva	185.0	1.6	3.3	10.4	31.1	53.6
				Serum	183.0	18.6	27.1	18.2	14.3	21.8
				Ratio	1.0	0.1	0.1	0.6	2.2	2.5
32(c).	F	20's	KI Toxicity	Saliva	153.0	2.0	5.2	14.3	31.6	46.9
				Serum	124.0	27.5	35.2	20.1	8.2	9.0
				Ratio	1.2	0.1	0.2	0.7	3.9	5.2
33.	F	20	Normal	Saliva	249.0	2.8	5.7	13.4	26.0	52.2
				Serum	125.0	28.9	33.8	19.7	10.4	7.3
				Ratio	2.0	0.1	0.2	0.7	2.5	7.2
34.	F	21	Normal	Saliva	158.0	1.7	5.4	10.6	22.6	59.7
				Serum	105.0	27.8	36.8	18.7	9.4	7.3
				Ratio	1.5	0.1	0.2	0.6	2.4	8.2
35(a).	F	30's	Normal	Saliva	84.0	5.6	7.2	12.3	26.0	48.9
				Serum	145.0	38.2	27.8	19.5	6.8	7.7
				Ratio	0.6	0.2	0.3	0.6	3.8	6.4

TABLE 6 cont.

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
35(b).	F	30's	Normal	Saliva	177.0	1.4	8.7	14.6	27.8	47.5
				Serum	145.0	38.2	27.8	19.5	6.8	7.7
				Ratio	0.8	0.1	0.3	0.9	4.0	6.0
36.	M	30's	Normal	Saliva	228.0	6.1	8.0	14.4	25.7	45.8
				Serum	150.0	29.1	30.8	20.1	9.4	10.7
				Ratio	1.5	0.2	0.3	0.7	2.7	4.3
37.	F	20's	Normal	Saliva	455.0	4.5	8.0	15.6	25.3	46.5
				Serum	152.0	30.6	36.0	17.6	8.0	7.7
				Ratio	3.0	0.2	0.2	0.9	3.2	6.0
38.	F	30's	Normal	Saliva	269.0	3.5	6.9	13.7	34.9	41.0
				Serum	150.0	19.1	29.0	22.0	13.7	16.2
				Ratio	1.8	0.2	0.2	--	2.5	2.5
39.	M	30	Normal	Saliva	210.0	3.9	8.6	15.7	26.7	45.0
				Serum	107.0	29.5	32.8	17.2	9.9	10.5
				Ratio	2.0	0.1	0.3	0.9	2.7	4.3
40.	F	21	Normal	Saliva	261.0	4.0	9.8	17.6	28.6	40.0
				Serum	132.0	29.2	32.0	20.8	10.8	7.2
				Ratio	2.0	0.1	0.3	0.9	2.6	5.6

Subject	Sex	Age	Diagnosis	Sample	Total	LD_1	LD_2	LD_3	LD_4	LD_5
41.	M	30's	Normal	Saliva	100.0	3.	8.8	13.5	24.1	47.3
				Serum	107.0	29.	32.8	17.2	9.9	10.5
				Ratio	0.9	0.	0.3	0.8	2.4	4.5
42.	F	20's	Normal	Saliva	102.0	3.8	6.1	12.8	27.1	50.2
				Serum	137.0	24.4	34.9	19.4	11.2	8.1
				Ratio	0.7	0.1	0.2	0.7	2.4	6.2
43.	F	20's	Normal	Saliva	172.0	5.1	7.4	12.4	24.8	48.3
				Serum	111.0	23.6	33.9	22.5	7.8	9.2
				Ratio	1.5	0.2	0.2	0.6	0.4	5.3
44.	F		Cystic Fibrosis	Saliva	100.	4.2	7.4	15.5	27.3	45.4
				Serum	--	--	--	--	--	--
				Ratio	--	--	--	--	--	--
			Cystic Fibrosis	Saliva	105.0	8.7	10.2	17.3	25.0	38.8
				Serum	--	--	--	--	--	--
				Ratio	--	--	--	--	--	--
	M	23	Cystic Fibrosis	Saliva	300.0	8.5	13.1	19.4	31.2	27.8
				Serum	--	--	--	--	--	--
				Ratio	--	--	--	--	--	--

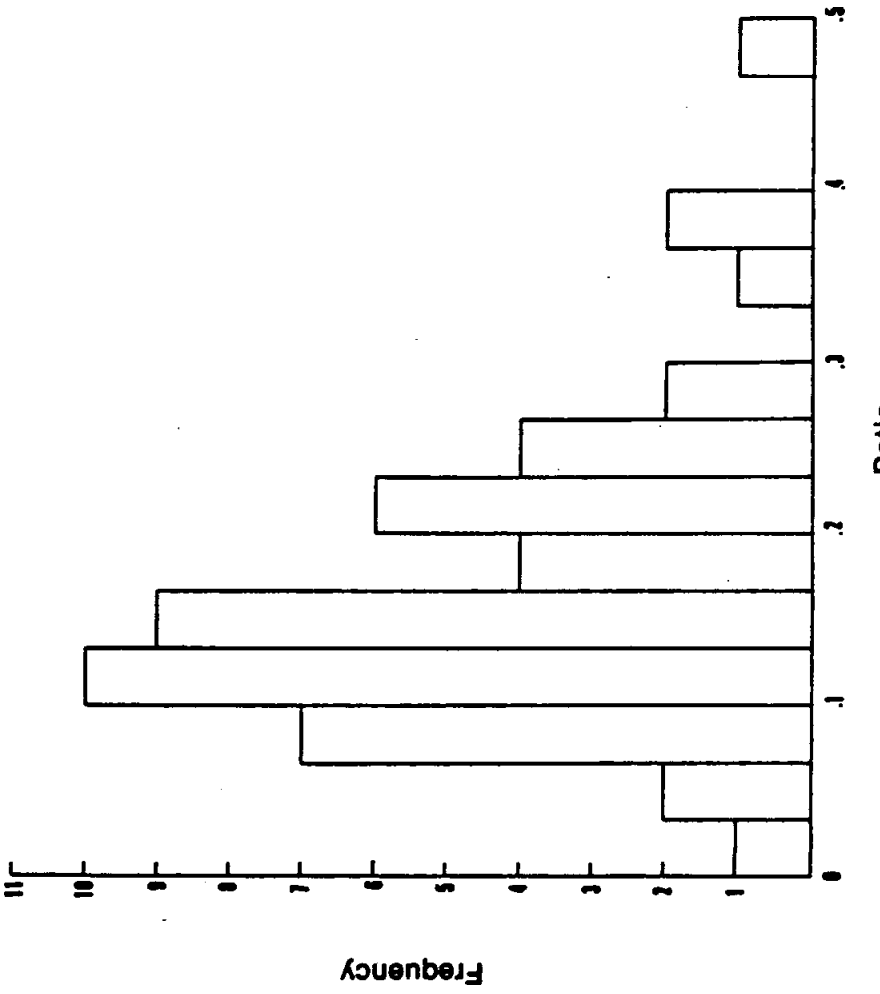


Fig. 9. LDH FRACTION 1 RATIO [Saliva/Serum]

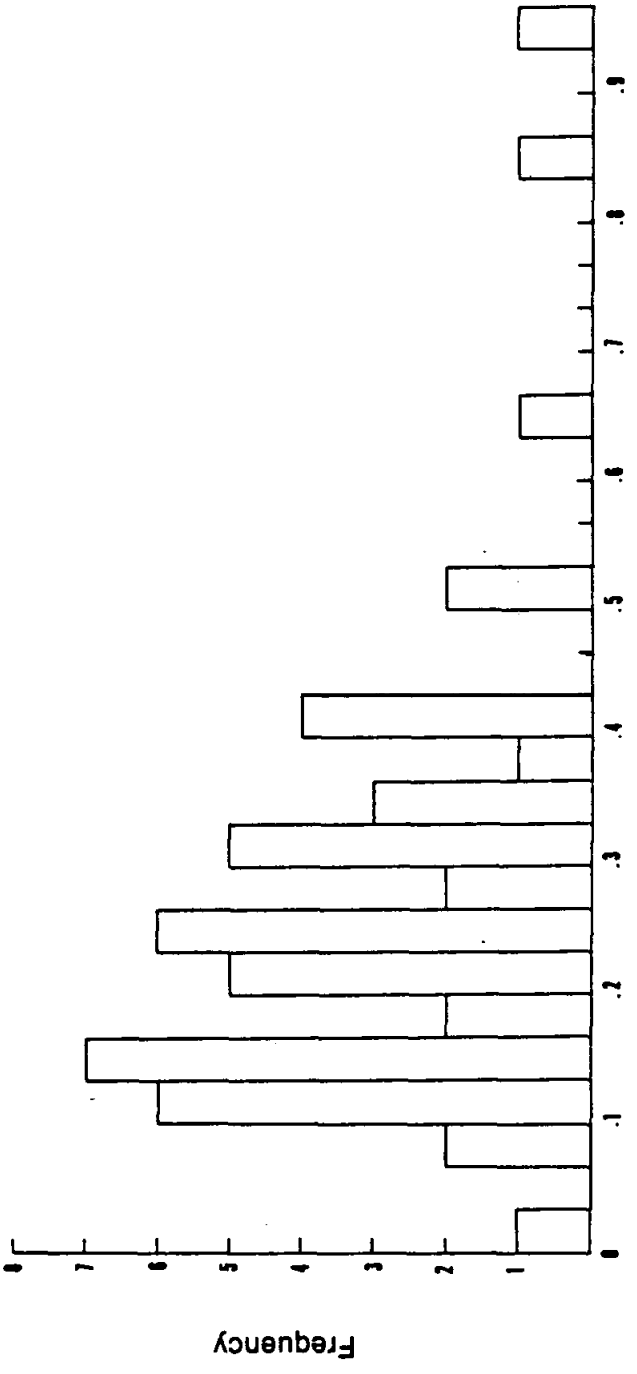


Fig. 10. LDH FRACTION 2 RATIO [Saliva/Serum]

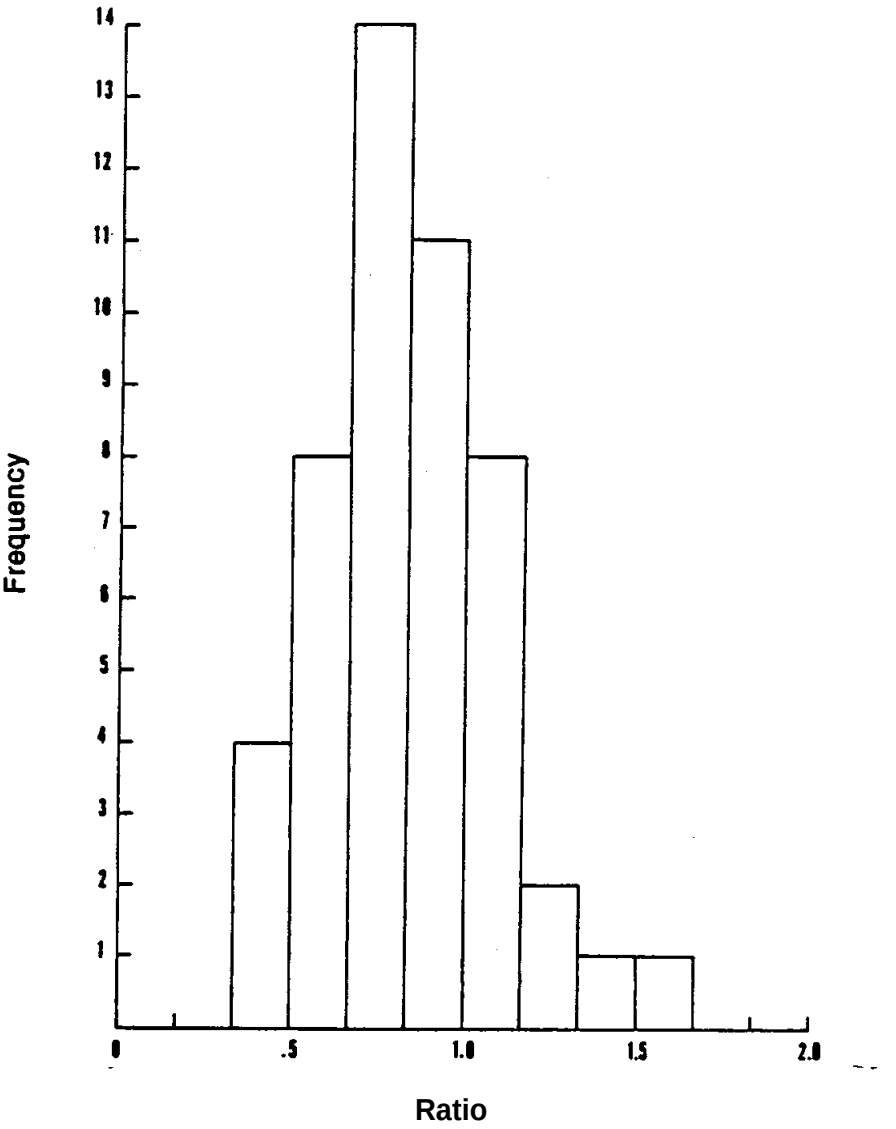


Fig. 11. LDH FRACTION 3 RATIO [Saliva/Serum]

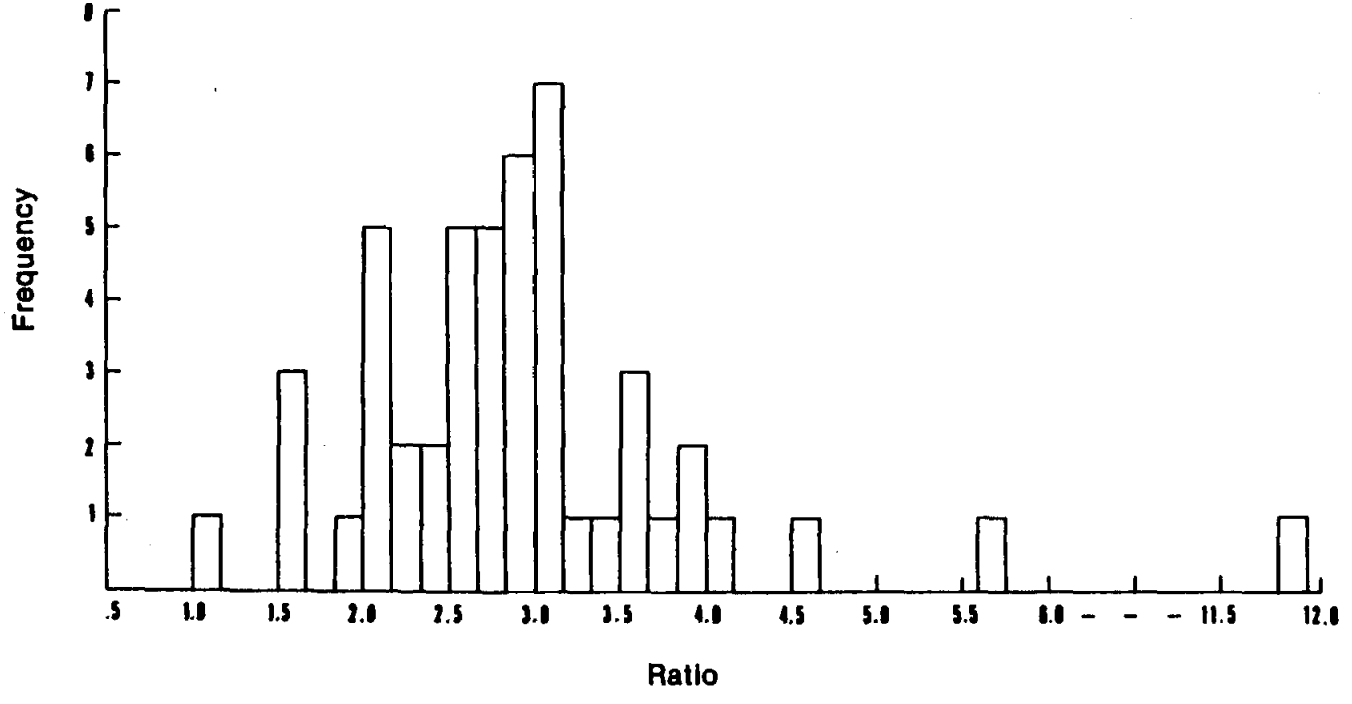


Fig. 12. LDH FRACTION 4 RATIO [Saliva/Serum]

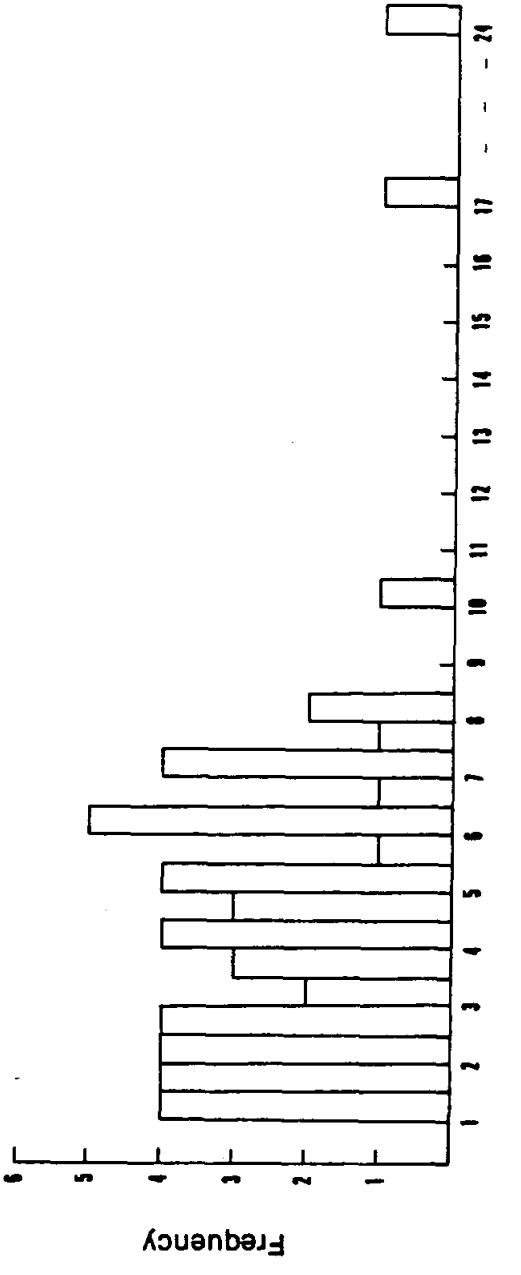


Fig. 13. LDH FRACTION 5 RATIO [Saliva/Serum]

Fraction one did form a tight histogram similar to a gaussian distribution with only one outlier. This outlier was only marginally removed from the main distribution. The diagnosis for this outlier was "possible gallbladder disease." The median frequency ratio was .175. The saliva/serum ratios for fraction two were more spread out along the graph varying from less than 0.1 to over 0.9 as opposed to fraction one which ranged from less than 0.1 to only 0.5. The median ratio value was .25.

The third fraction also formed a narrow distribution with values from 0.30 to 15. The median value was 0.7.

Fraction four was very uniform in its distribution only the ratio axis. The ratios ranged from 1.0 to 4.52, with a median value of 2.8. These were two significant outliers of 5.56 and 11.9. The 5.56 outlier was a female subject in her 60's with a diagnosis of "coronary insufficiency." The 11.9 ratio value was a subject diagnosed with thrombus arthritis.

The fifth fraction had a range of ratios from 1 to 8, with a median value of 4.9. There were two significant outlying values for this fraction. The ratios of 17.0 and the 23.6 were both from the same subject. The diagnosis was coronary insufficiency.

Ratios between the total LD concentration of saliva and serum are listed in Table 5. There is apparently little correlation of these totals to each other.

The author, as a control subject, provided a total of eighteen samples representing morning and late evening samples. The morning samples were collected at approximately 7 A.M. and the evening samples at 10 P.M. In addition, samples were collected both before and after stimulation as described earlier.

Some observations can be made concerning the control results: 1) The morning samples were consistently much higher in total LD enzymatic activity than the evening samples. This was true for stimulated and unstimulated samples. However, there was no correlation between the isoenzyme distribution and the time of day when the sample was taken. 2) Stimulation had a profound effect on the total LD activity, and a less conspicious effect on the distribution of the isoenzymes. Total activity was consistently much higher in the unstimulated sample. These results suggest that little significance can be attached to total LD activity in saliva. The isoenzyme distribution patterns were generally very similar for both type of samples, although LD_5 was usually higher, and LD_1 - LD_4 lower, in the stimulated samples. Table 7 shows the results of the stimulated and unstimulated data.

A statistical analysis of each of the isoenzyme fractions for the composite of subjects (55 samples) and for 9 control subject samples are compared in Table 8.

The coefficients of variations for the stimulated and unstimulated control subjects were considerably lower

TABLE 7

RESULTS OF CONTROL SUBJECT'S A.M. AND P.M. SALIVA SAMPLES BEFORE AND AFTER STIMULATION IN mU/mL FOR TOTAL LD AND THE LD FRACTIONS IN PERCENTAGES

	Before Stimulation						After Stimulation					
Time	Total	LD_1	LD_2	LD_3	LD_4	LD_5	Total	LD_1	LD_2	LD_3	LD_4	LD_5
PM	177	6.8	10.5	15.0	26.2	41.5	47	3.3	6.2	10.5	23.7	56.2
AM	762	8.0	12.8	16.9	26.8	35.4	173	2.5	5.9	11.0	24.0	56.6
PM	471	6.1	10.4	15.3	29.7	38.5	97	4.9	7.2	13.9	22.5	51.5
AM	796	3.4	9.3	17.5	26.4	43.3	138	4.9	7.3	10.9	23.1	53.7
PM	135	2.9	10.4	15.8	25.0	45.9	70	2.7	7.6	13.5	26.2	52.0
AM	886	2.7	10.6	18.3	31.3	37.0	167	2.6	6.2	14.9	28.9	48.4
AM	920	4.4	12.5	19.2	29.2	34.6	203	4.3	8.1	14.0	24.5	49.0
PM	291	5.4	10.9	16.9	26.4	40.3	170	4.0	8.3	14.2	25.2	49.3
AM	821	6.6	9.6	16.2	26.0	41.7	240	4.5	8.3	12.3	28.9	48.0

TABLE 8

STATISTICAL DATA FROM COMPOSITE OF SUBJECTS
COMPARED WITH THAT OF CONTROL SUBJECT

	(55 Samples) Composite					(9 Samples) Control Before Stimulation				
	LD_1	LD_2	LD_3	LD_4	LD_5	LD_1	LD_2	LD_3	LD_4	LD_5
Mean	4.3	7.9	15.1	27.4	44.5	5.0	10.8	16.9	27.4	39.8
Standard deviation	2.1	2.8	3.9	4.8	8.4	2.1	1.2	1.5	2.1	3.7
Coefficient-of-variation (%)	50.0	35.9	26.0	17.3	18.9	41.2	10.9	9.2	7.6	9.4

	(9 Samples) Control After Stimulation				
	LD_1	LD_2	LD_3	LD_4	LD_5
Mean	3.7	7.2	12.9	25.2	51.6
Standard deviation	1.0	0.9	1.6	2.4	3.2
Coefficient-of-variation (%)	26.3	13.0	12.0	9.3	6.1

than the composite group for each fraction. The coefficient-of-variation between the stimulated and unstimulated control subject showed variances from fraction to fraction. The LD_1 and LD_5 had considerably lower C.V.'s for the stimulated. However, for LD_2, LD_3, LD_4, the C.V.'s were moderately lower for the unstimulated.

Parotid Fluid

The samples of parotid fluid were analyzed the same as the previous saliva samples. They were assayed for <u>total</u> LD enzymatic activity and then electrophoresed on cellulose acetate to study their isoenyzme patterns. An important difference was observed between the results of the mixed, whole saliva patterns and that of the parotid samples. Unlike the whole saliva samples, the parotid did not show the "reversed" pattern observed with all whole saliva samples, but showed an electrophoretic pattern similar to blood serum samples. The illustration (Figure14) demonstrates the patterns obtained from parotid fluid.

Another difference was observed between the parotid fluid and the whole saliva samples. This difference was with respect to the <u>total</u> enzymatic activity. The parotid fluid sample contained considerably less LD total activity than most whole saliva samples. The parotid samples' concentrations from canalization were 12 and 35 mU/mL respectively. The standardized collections on the control for whole saliva

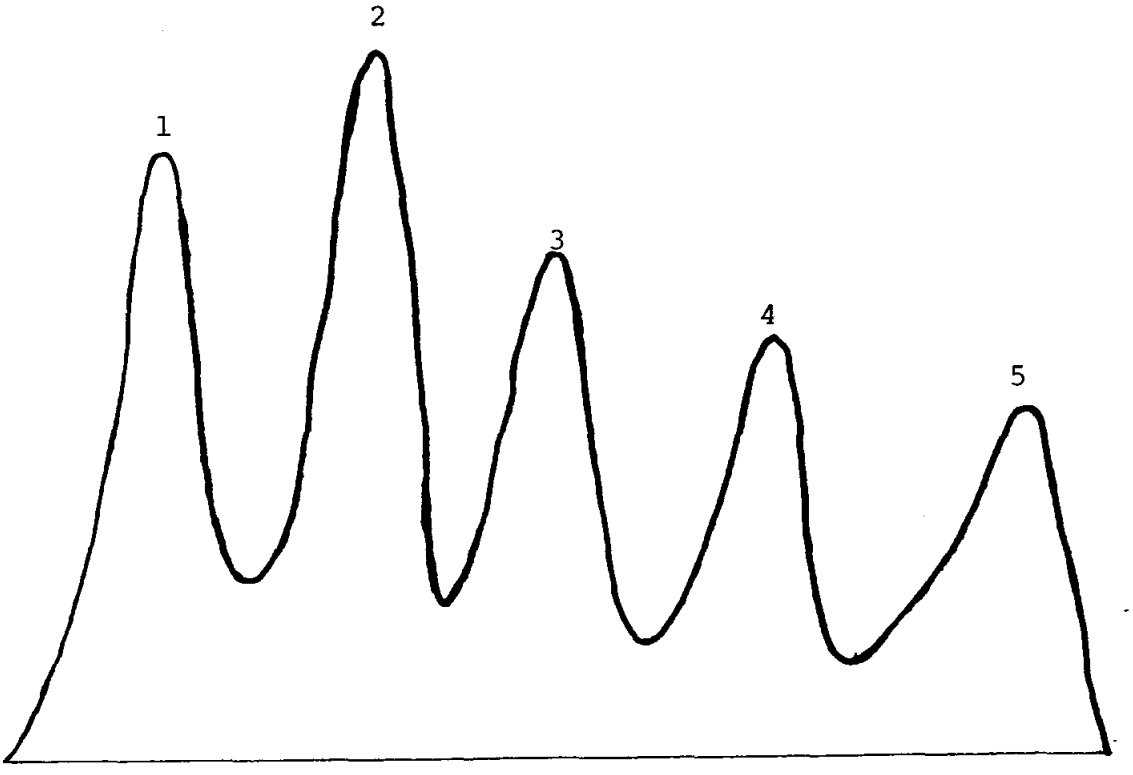


Fig. 14. Electrophoretic Pattern of Parotid Fluid Obtained by Canalization.

samples were assayed in order to have a reliable comparison of total LD concentrations with parotid totals. Total LD's were run on whole saliva samples collected without stimulation and after stimulation. The results are listed in Table 9. As mentioned above, the stimulation of saliva flow changed the concentration of the total LD enzyme activity. The concentrations were always higher before stimulation (from 2 to 10 times higher). The total concentrations of parotid fluid were, in most cases, considerably lower than either the stimulated or unstimulated whole saliva samples.

TABLE 9

TOTAL LD CONCENTRATION COMPARISON ON ONE SUBJECT

Before Stimulation	After Stimulation	Parotid Samples (Stimulation)
85	65	12 (canalization)
228	27	35 (canalization)
151	68	10
131	50	10
216	70	5
113	96	12
158	65	10
224	97	
203	70	

CHAPTER 4

DISCUSSION AND CONCLUSIONS

The only report found in the chemical literature attempting measurements of LD in human saliva is the paper by Kubicz, et al., "Lactate Dehydrogenase Isoenzymes in Three Types of Human Saliva."[7] In the study, the authors used disc electrophoresis to separate LD isoenzymes of human saliva. The investigations were done on mixed saliva and on saliva from the submandibular-sublingual glands and parotid glands. Twelve persons ranging from 19 to 26 years and apparently healthy were used.

Albumin was measured on the saliva samples using a micrometer. The samples were separated using disc electrophoresis, at pH 9.4, 5.5% polyacrylamide gel, and at 4°C. temperature. Depending on the concentration of albumin in the saliva, they added saccharose to the samples to achieve a final 20% concentration.

After the electrophoresis, they detected the presence of isoenzymes with the aid of specific dyes. The gels were then incubated in the dye-bath from 15 to 60 minutes, in darkness.

In their discussion, Kubicz, et al. stated they did not assay the saliva samples for <u>total</u> LD, but depended upon the albumin concentration to regulate the quantity of matter

undergoing electrophoresis. They added 15 mg of albumin to the discs in order to produce a clear isogram.

They indicated that LD_2, LD_3, and LD_4 prevailed in mixed saliva, and in saliva from the submandibular-sublingual glands and parotid glands. The differences between different types of saliva were observed mainly with respect to LD_5, which was not found in the saliva from the submandibular-sublingual glands while it was evident in the remaining types of saliva. They state that their investigations demonstrated that a part of LD activity in mixed saliva is of endogenous origin.

The results reported by Kubicz, et al. were not in agreement with the results of this investigation. They stated that the hybrid isoenzymes LD_2, LD_3, and LD_4 predominated in all types of saliva. I found the isoenzyme LD_5 to be predominate for mixed saliva samples in over 50 subjects. They also stated that the parotid saliva has a predetermined predominance to type M isoenzyme (this refers to the LD_5 isoenzyme). I found the parotid samples to have patterns similar to blood serum samples, i.e., LD_5 not being the predominant type.

The difference in results between the two studies may be attributed in part to the experimental methods used. The exact technique Kubicz and coworkers used to obtain the samples from the oral cavity is not explained, although the article did mention that there were paddings and sterile compresses used to segregate the salivary glands. The exact

technique for collection for this study was delineated earlier. Kubicz, et al. also indicated that in order to demonstrate the presence of LD isoenzymes in some samples, they had to concentrate the volumes to 0.1 to 0.2 ml by evaporation. They also introduced other variables in the sample by adding albumin to enhance the reaction, saccharin to compress albumin, and prolonging the time of reaction of the enzymatic development gel for several hours in some cases. The electrophoretic method was a disc gel technique.

Conversely, I did not have to concentrate any samples before carrying out the analysis. Also I did not add any reagents to the samples for pre-treatment purposes. The actual electrophoresis was carried out on cellulose acetate in this study. The volumes I obtained from subjects were adequate each time so that the only pre-treatment was centrifugation to remove any particulate matter. This also applies to the parotid samples which Kubicz, et al. indicated they had difficulty obtaining enough sample from one person to carry out analyses. In fact, they mentioned they had to increase the separation process by adding 500 mg of albumin to demonstrate LD activity. They did not actually analyze their samples for total LD activity. In the present study there was not any question concerning LD activity since all samples were first analyzed for total enzymatic activity before electrophoresis.

In further comparison of this research with the Polish study, the following comments may be made.

Kubicz, et al. did not try to relate their study with any clinical significance. They stated that their 12 subjects were all apparently healthy between the ages of 19 to 26 years in age. Their study emphasized the effort to identify the types of **LD** isoenzymes in three kinds of saliva (mixed saliva, saliva from submandibular glands and under-the-tongue glands, and saliva from parotid glands).

In this study the author used over fifty subjects ranging in age from 16 to 87 years. The emphasis was not on proving whether or not the five **LD** isoenzymes exist in saliva, but to objectively report the findings of saliva isograms along with the patterns of blood samples on these same subjects and to ascertain whether or not a statistical analysis of the data would reveal a trend or be related to some clinical significance. To make this meaningful, both apparently healthy subjects and subjects with a diagnosed disease were used.

In addition, the Polish paper did not include any information that indicated they performed any parallel testing of their subjects' blood isoenzyme patterns. In fact, no quantitative data were reported.

The last observation in comparing the two studies is that of the effect stimulation may have on the **LD** isoenzyme concentrations or patterns. Kubicz's group did not mention this effect, but it was an aspect taken into consideration in this study.

Summary of Conclusions

1. The results of over one hundred whole saliva electrophoretic patterns from over 70 subjects (53 reported in Table 6) showed that there is a progressive pattern of the LD isoenzymes with the LD_1 fraction having the lowest concentration and the next four fractions increasing in concentration respectively. This general pattern is different than that of blood serum.

2. There is no apparent distinguishable difference in any of the whole or mixed saliva LD patterns attributable to a specific disease state. The occasional outlier could not serve as proof of any conclusive departure from the other patterns.

3. There is a marked difference between the total enzymatic activity of whole saliva and that of parotid fluid. The parotid samples are considerably lower in activity than the whole saliva samples.

4. Parotid fluid has an LD electrophoretic pattern entirely different than that of whole saliva. While whole saliva electrophoretic patterns always show a concentration progression from LD_1 to LD_5, the parotid fluid patterns are similar to blood serum.

5. There is a strong dependence of total LD enzymatic activity on the stimulation of saliva flow. The samples collected after stimulation are always considerably lower in activity than before stimulation.

6. The results of the control subject and the composite group comparisons showed that there are real variations in isoenzyme distribution between different individuals, greater than the normal diurnal variation to be expected for one person.

From the C.V.'s obtained from the above comparisons, it seems feasible that some expected values can be established for an individual subject.

7. The ratios obtained for saliva/serum did not show any apparent clinical significance.

8. The similarity between the isograms of parotid saliva and blood serum strongly suggest that an equilibrium exists between the enzymes in these two fluids.

BIBLIOGRAPHY

1. Tietz, N., Ed. Fundamentals of Clinical Chemistry. 2nd. ed. W. B. Saunders Co., Philadelphia, Pa., 1976, pp. 565-693.

2. LDH Electrophoresis Procedure No. 6, April 1981. Helena Laboratories, P.O. Box 752, Beaumont, Texas 77704.

3. Lott, J. and Stang, J. M. Serum Enzymes and Isoenzymes in the Diagnosis and Differential Diagnosis of Myocardial Ischemia and Necrosis. Clinical Chemistry 26, 1241-1250 (1980).

4. Levine, M. J. and Ellison, S. A. Immuno-Electrophoretic and Chemical Analyses of Human Parotid Saliva. Archs Oral Biology Vol. 18, pp. 839-853 (1973).

5. Danhof, M. and Breimer, D.D. Therapeutic Drug Monitoring in Saliva. Clinical Pharmacokinetics 3:3957 (1978). Adis Press (1978).

6. Mandel, I.D. and Ellison, S.A. The Proteins of Human Parotid and Submaxillary Saliva. Annals New York Academy of Sciences, 106, 271-277, 1963.

7. Kubicz, A.; Kuczyinska-Koziowska, S.; Wolalnska, L. Lactic Dehydrogenase Isoenzymes in Three Types of Human Saliva. Czas Stomatol, Feb. 34 (2): 121-5, 1981.

8. Wotman, S. and Mandel, J. D. The Salivary Secretions in Health and Disease. Saunders Co., Philadelphia, Pa., 1976.

9. Mason, D. K. and Chisholm, D. M. Salivary Glands in Health and Disease. Saunders Co., Philadelphia, Pa., 1975.

10. Shaw, J., et al. The Chemistry and Physiology of Saliva, Textbook of Oral Biology. W. B. Saunders Co., Philadelphia, Pa., 1978, pp. 593-616.

11. Shugar, D. Enzymes and Isoenzymes; Enzymes and Isoenzymes Structure, Properties and Function. Academic Press, London and New York, 1970.

12. Rosalki, S. B. Diagnostic Enzymology, 2nd Edition. Dade Reagents, Miami, Florida (1969).

13. Leung, Y. and Henderson, A. Thin-Layer Agarose Electrophoresis of Lactate Dehydrogenase Isoenzymes in Serum: A Note on the Reporting and on the Lactate Dehydrogenase Isoenzyme-1/Isoenzyme-2 Ratio in Acute Myocardial Infarction. Clinical Chemistry 25/2, 209-211 (1979).

14. Dito, W. R. A Simple Time-Saving Method for Interpretation Report Generation: 1. Lactate Acid Dehydrogenase Isoenzymes. American Journal of Clinical Pathology 59: 439-447, 1973.

15. Wilkinson, J. H. Isoenzymes. J. B. Lippincott Company, Philadelphia, Pa., 1965, pp. 43-79.

16. Everse, J. and Kaplan, N. Lactate Dehydrogenases: Structure and Function. Advances in Enzymology, Vol. 37, pp. 61-133 (1973).

REFERENCES

Beaty, H. N. and Oppenheimer, S. Cerebrospinal-Fluid Lactate Dehydrogenase and its Isoenzymes in Infections of the Central Nervous System. The New England Journal of Medicine, Vol. 279 No. 22, 1197-1202 (1968).

Harrison, T. R. Principles of Internal Medicine, 9th Edition. McGraw Hill, New York, pp. 1233-1234 (1980).

Maxwell, M. H. and Kleeman, C. R. Clinical Disorders of Fluid and Electrolyte Metabolism, 3rd. Edition. McGraw Hill, New York, pp. 1578-1579 (1980).

Race, G. J. Body Fluids, Including Urine, Laboratory Medicine, Vol. 4. Harper and Row, pp. 6-7 (1976).

MacKay, C.; Abramson, D.; Ellsworth, R.M.; Kitchen, F.D.; and Michael P. Lactate Dehydrogenase in Tears. American Journal of Ophthalmology 90:385-387 (1980).

Bhoola, K.D.; McNicol, M.W.; Oliver, S.; and Furan, J. Changes in Salivary Enzymes in Patients with Sarcoidosis. The New England Journal of Medicine Vol. 281 No. 16 (1969), pp. 887-879.

Wroblewski, F.; Decker, B.; and Wroblewski, R. The Clinical Implications of Spinal-Fluid Lactic Dehydrogenase. Activity. The Journal of Medicine, Vol. 258 No. 13, 635-639 (1958).

Beeley, J.A. and Chisholm, D. M. Sarcoidosis with Salivary Gland Involvement: Biochemical Studies on Parotid Saliva. J. Lab Clinical Medicine Vol. 88 No. 2, pp. 276-280 (1975).

Fischer, C. J., et al. Sjogrends Syndrome. Electrophoretic and Immunological Observations on Serum and Salivary Proteins of Man. Archs Oral Biology Vol. 13, pp. 257-270 (1968).