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AN INVESTIGATION OF A POSSIBLE CORRELATION BETWEEN METALLIC ELEMENTS IN HUMAN HAIR AND CORONARY HEART DISEASE

A Thesis
Submitted to the Faculty
of
Western Michigan University

Kalamazoo, Michigan

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by

Lamar E. Bullock
June, 1962

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Finally, he wishes to acknowledge the assistance and abiding faith of his wife, Pat, during the many months of this work.

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INTRODUCTION

In the academic year 1959-1960 the National Science Foundation supported an undergraduate research program at Western Michigan University for the spectrographic analysis of human hair. This preliminary work concerned itself with determining the quantities of magnesium, iron, and calcium in human hair. The hair samples were taken from a supposedly normal cross section of people. This group consisted of 41 women and 52 men. The ages varied from 2 to 72. Most of the hair samples were collected from a local barber shop and beauty shop, although a small portion of the samples was collected from individuals. Information concerning the individual's sex, age and race were recorded along with the color of the hair, for each sample received.

The analysis of the samples revealed nothing unique or distinctive concerning the quantities of calcium and iron. With few exceptions, however, the magnesium content of the samples for the men was lower than for the women. This disparity in magnesium content appeared to increase after age 50. In searching for possible explanations for the magnesium data, attention focused on the inequality of heart ailments in men and women.

Arteriosclerosis in particular is thought to be related

to the elements magnesium and calcium. 1

This is a report of the research which was undertaken to investigate a possible correlation between coronary ailments and the metallic content of human hair.

CHAPTER I

DEVELOPMENT OF THE PROJECT

The possibility of the existence of a relationship between coronary ailments and body metals was supported by a publication of the Public Health Service.² This study concerned the coronary death rates for 163 metropolitan areas across the United States. A letter was sent to the Public Health Service asking for more information on this study and inquiring whether information on the metallic content of the water supplies in these same cities was available. The Public Health Service supplied a copy of the full report and indicated that information concerning the water supplies was available in material published by the United States Geological Survey.³

In comparing the coronary death rates with the magnesium content of the water supplies for various cities it was found that there was not a clear distinction between magnesium content and the death rate for those cities with high death rates. However, the cities with the low death rates appeared to have higher magnesium content in the water, (see Figure 1).

This correlation was supported by Schroeder⁴ in his statistical investigation of the same data. Schroeder

MAGNESIUM CONTENT OF TREATED WATER SUPPLIES VS

DEATH RATE FROM CORONARY HEART DISEASE FOR WHITE MALES AGED 45 TO 64

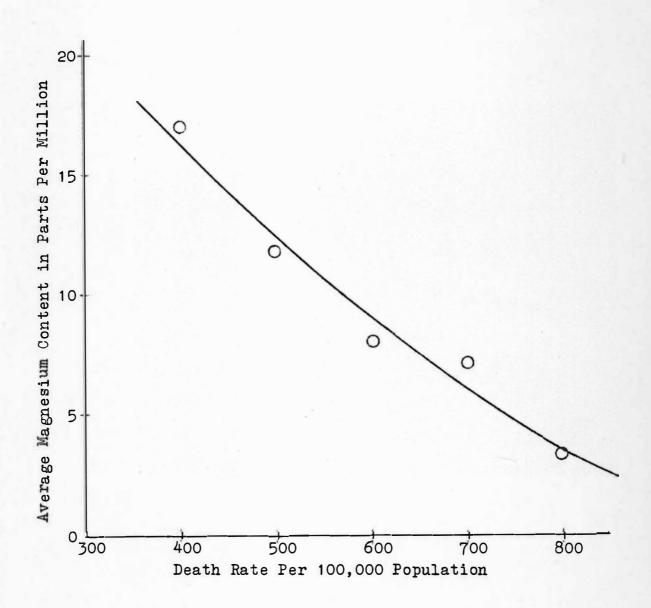


FIGURE 1

also investigated many other aspects of the treated water supplies of these 163 municipalities. He stated that he had found significant negative correlations: (i.e., high value corresponds to low death rate) for the following characteristics, magnesium, calcium, bicarbonate, sulfate, fluoride, dissolved solids, specific conductance and pH. Mention was made of the fact that acidic or soft waters had a tendency to dissolve certain minerals such as lead, zinc, and copper out of the pipes. Schroeder's report also pointed out the fact that these correlations did not exist for non-cardiovascular causes of death. This was believed to support the correlations drawn in the case of the coronary death rates.

The final development, that indicated this project might produce fruitful results, came when the project was well under way. This was a report of a speech given by Dr. William H. Strain of the University of Rochester School of Medicine. The article indicated that vanadium and zinc are keys which Dr. Strain believes are linked to a wide range of diseases. More specifically, it stated a possible connection between these elements and arteriosclerosis. He also noted the fact that certain geographical areas in the United States have a much higher rate of heart disease than do other areas.

The above facts are not enough to state that there is a definite relationship between the metallic content of a person's body and his chances of having coronary ailments. They are, however, enough to indicate that further investigation might produce results which would prove interesting to medical science.

CHAPTER II COLLECTION OF HAIR SAMPLES

While accumulating some of the previously mentioned data, an attempt was made to obtain coronary hair samples by various methods, all of which failed. In order to get a sufficient supply of samples, it was necessary to enlist the support of area doctors. A letter (see Appendix I) was sent to all the doctors listed in the Kalamazoo telephone directory. This letter contained a brief description of the project and solicited the assistance of the doctor for the collection of samples. The method of sample collection outlined to the doctors would not disclose the identity of the patient, unless the patient personally desired to obtain a report of the findings.

Out of the 120 doctors that were sent requests,

42 returned the enclosed self addressed post card which
was provided. Of these, 18 indicated they were treating
heart patients and would be willing to help. The other
24 stated that they did not have heart patients and
were therefore unable to help.

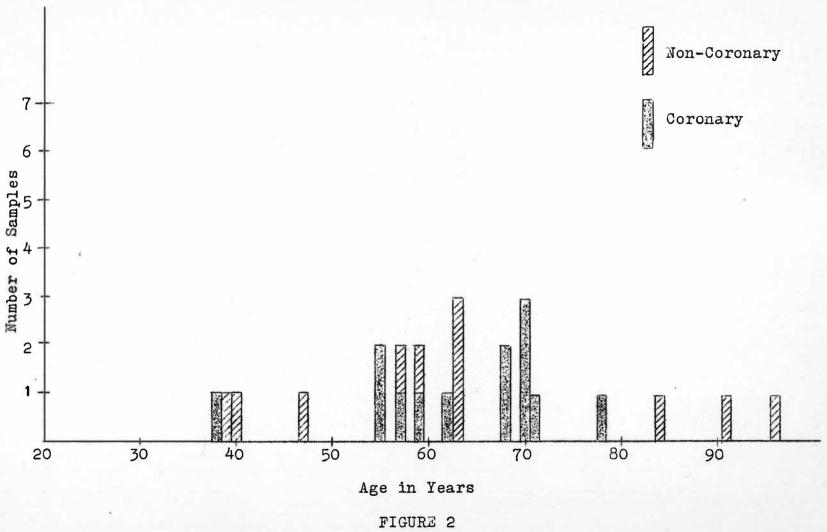
It was then necessary to deliver the patients' letters (see Appendix II) to the individual doctors for them to address and send to their coronary patients.

In all, 205 letters were delivered to doctors out of which 61 hair samples were received. Of these, 47 enclosed names and addresses indicating the desire to be informed of the progress in our research.

In order to compare the metallic content of hair from the coronary patients with that from non-coronary patients, it was necessary to analyze the non-coronary patients by exactly the same method and at the same time as the coronary patients. This was done by selecting samples from our previous supply that corresponded as nearly as possible to the coronary patients in the matter of age and sex. When it was found that male hair samples were needed, the barber shop was again called upon to collect the samples from men over 50.

Figure 2 shows the distribution, by age, of female samples for both the coronary and the non-coronary groups. Figure 3 provides the same information for male samples. More female samples are desirable but the male samples are adequate to observe any significant differences between the coronary and non-coronary samples.

DISTRIBUTION OF FEMALE SAMPLES



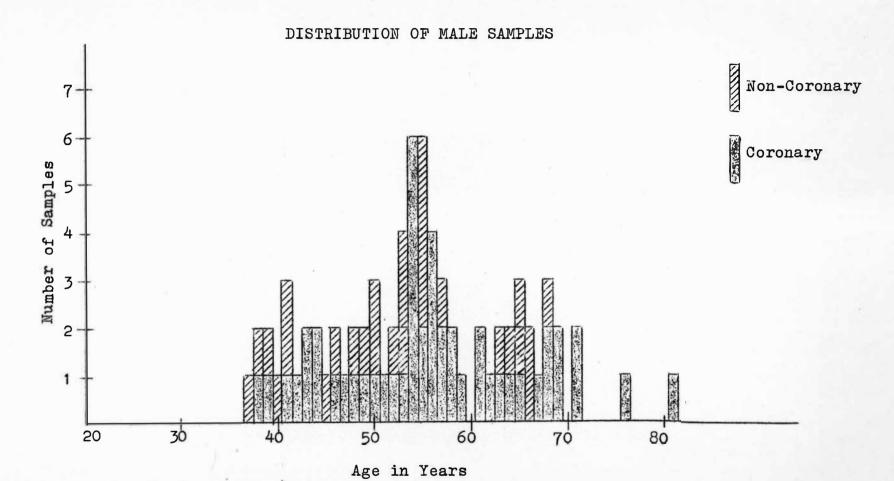


FIGURE 3

CHAPTER III

SAMPLE PREPARATION

When the hair samples were collected, it was necessary to give them extensive preparation before the spectrographic analysis could be made. This preparation included washing, weighing, ashing, and drying the samples on the surface of electrodes.

Throughout all but the last step of the preparation, the samples were contained in individual sample bottles (see Figure 4). These bottles are about one inch in diameter and hold approximately 0.5 fluid ounces. Before the samples were put in the bottles, each bottle was given an identifying number using a Burgess Vibra-Tool. The bottles were then washed thoroughly with a detergent solution and rinsed successively in tap water, nitric acid, and distilled water. They were then dried on a hot plate and placed in a desiccator for about two days before a tare weight was taken. The tare weights on the bottles ran about 16 grams.

After the bottles had been properly tare weighted, the samples were placed in the bottles and the proper identification was made. Each bottle was then covered with a square of gauze, which was held in place with a rubber band, in preparation for the washing procedure.

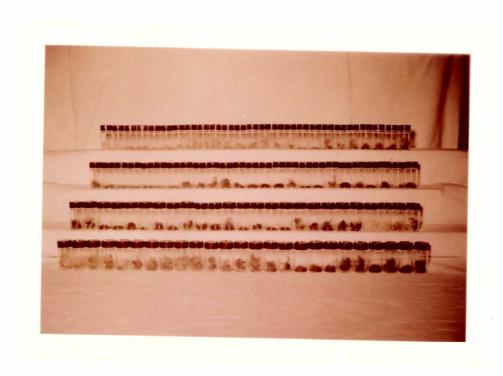


FIGURE 4

Photograph of the hair samples showing the gradation in color and grayness. Starting in the upper left the first 71 bottles are arranged in order from all gray down through samples with just a trace of gray. The last 29 bottles show the gradation of color from blonde through black.

The gauze was used to facilitate the washing procedure without the loss of hair. A detergent (Alconox) was used to wash the hair. This washing was done by pouring the detergent into the bottles through the gauze. The detergent and hair were then shaken vigorously by hand. Next, the hair was rinsed several times in tap water and the washing step was repeated. Then the samples were rinsed in tap water until there was no sign of the detergent left. After this, the hair was rinsed several times in distilled water. It was then drained dry and placed on a hot plate, at about 75° C, to dry thoroughly. The samples were then stored in a desiccator for about two days prior to weighing.

All the weighing of the samples and bottles was accomplished on a single pan Ainsworth "Right-A'Weigh" balance. The least count of this balance was 0.0001 grams and all the samples were weighed to 0.1000 grams \pm 0.0003 grams. An additional error in the weighing was encountered because the hair picked up moisture during the time of weighing. Nevertheless, the sample weights were maintained within a \pm 1% range. During the weighing, the samples were handled with tweezers and in some cases it was necessary to cut the samples with a pair of small scissors to simplify handling. Both of these instruments were cleaned before each use.

After the samples had been weighed to 100 mg, it was necessary to reduce them to an ash. That is, the unwanted organic substance had to be removed from the hair to reduce its bulk. The best agent for dissolving the samples was nitric acid. Other acids such as hydrochloric and sulfuric were unsatisfactory because they did not completely dissolve the samples. The ash left, after the nitric acid hair solution was taken to dryness, still contained a large amount of carbon, which it was desirable to remove. Previously, this was done by igniting the ash in an oxygen atmosphere, but this method had limitations. It required an additional handling of the sample and the possibility existed that a portion of the sample would be lost by being carried away with the rising gases. A better method of oxidizing the excessive carbon was developed using perchloric acid. The basic plan for perchloric oxidation was outlined by G. Frederick Smith⁶, but the details for this type of sample had to be determined by experimentation.

Since great care had to be exercised not to contaminate the samples, spectrographic tests were run on the nitric acid, perchloric acid, and distilled water to see whether or not they had an excessive amount of metallic elements. It was found that all three had more Mg and Ca than was desirable. A search was made,

therefore, for purer reagents. It was found that although Dupont reagent grade nitric acid was the purest available it still did not have the required purity. It was therefore decided to try purifying the acid by distillation. The distillation was performed in an all glass still and the distillant was checked frequently to determine its purity. Even though the glassware was thoroughly cleaned and rinsed in concentrated nitric acid, it was found that nearly as much contamination was picked up from the glass as was removed by the distillation. Even with these difficulties, the nitric acid was purified to a tolerable level by two distillations. The nitric acid distilled at a constant boiling temperature of 116° C.

Mallinckrodt perchloric acid was found to be purer than any of the other perchloric acids tested, but its magnesium and calcium level was high enough so that it was deemed desirable to try purifying it by distillation. Since perchloric acid has a reputation for being somewhat unstable, extra precautions were taken during its distillation. While the nitric acid was distilled in a hood with the normal safety glass doors, an extra safety glass shield was placed between the still and the doors during the distillation of the perchloric acid. The perchloric acid was found to distill at a constant boiling temperature of 195°C after the excessive water was

driven off. At no time was any violent or dangerous reaction observed. This distillation process seemed to reduce the perchloric contamination slightly, but the improvement was scarcely worth the effort involved in the distillation process.

The contamination in the distilled water was of most concern because a proportionally large amount of it was to be added to the samples. After some experimentation, it was discovered that cycling the distilled water through a Barnstead mixed bed deionizer was the best and easiest way of purifying the water. The contamination was taken to a very low level by this method.

After the different reagents were purified, it was necessary to determine how much of each was necessary to ash the sample. Since an automatic pipette was available which would deliver a constant 10 ml of solution every time, it was desirable to add this amount of solution to each sample. Tests showed that 1 ml of nitric acid and 0.5 ml of perchloric acid were sufficient to completely ash the test samples when added in a 10 ml solution. The final amounts decided upon were: 1.2 ml of nitric acid and 1.0 ml perchloric acid. The extra perchloric and nitric acid was used in anticipation that some of the samples might prove to be harder to oxidize

than the test samples.

It was also necessary to add a known and constant amount of internal standard to each one of the samples for reasons that will be explained later. Molybdenum was chosen for this internal standard and sufficient ammonium molybdate was introduced into the stock solution to cause 0.003 mg of molybdenum to be added to each sample.

The actual ashing procedure was carried out in a hood because the fumes from this reaction were abundant and toxic. The automatic pipette described above was used to add 10 ml of solution to each of the samples. It was then necessary to place the bottles on a hot plate, since the dissolving and oxidation of the hair at normal room temperature for these acid concentrations occur very slowly. The surface of the hot plate used was 12 in. x 20 in., which was large enough to hold all 107 sample bottles plus a row of empty bottles around the outside to help keep the heating as uniform as possible. (See Figure 5). The temperature of the hot plate was maintained between 90° C and 110° C so that the samples would steam but not actually boil. As the samples heated up and started to dissolve, the originally clear solution began to take on color. The color varied from a pale yellow to a deep brown. As the water was evaporated away, the samples dissolved further. The



FIGURE 5

A photograph of the samples dissolving in bottles on the hot plate. The bottles with the caps on are empty bottles used to keep the heating as uniform as possible.

samples were completely dissolved when the volume of the solution had been reduced to about 1/4 of its original volume. By the time the volume was down to about 1 ml, the solutions had cleared again.

Previous work during the N. S. F. project indicated that the ash weight of the women's hair was significantly higher than the men's. It was therefore desirable to weigh the ash of each one of the samples to confirm this result and to look for any other correlations. was previously indicated, it was difficult to weigh accurately to one-tenth of a milligram because of water vapor from the air condensing on the bottles. When first weighing the hair samples, the water vapor was only a fraction of one per cent of the unashed sample weight, thus it was not a problem. When weighing the ash, however, the water vapor introduced a much larger percentage of error as the ash only weighed about 2 mg. To minimize this, a drying machine was used. machine consisted of four cylinders where the sample bottles could be placed to be simultaneously heated, evacuated and dried by desiccant. When bottles were removed from the desiccator and placed in the drier, they reached an equilibrium dryness condition in about 10 minutes. Thus each of the samples was dried for 10 minutes and weighed immediately after removal from the

drier. When the samples were removed from the drier and placed on the balance pan, they picked up moisture at a measured rate of approximately 1 mg per minute. By recycling 14 of the samples at various times it was found that the weights could be reproduced with an average deviation of \pm 0.3 mg. After the samples were removed from the bottles, the bottles were washed and reweighed in the same manner.

To perform the spectrographic analysis, the sample had to be placed on the surface of carbon electrodes. The electrodes used were Ultra Purity Spectroscopic Graphite Electrodes obtained from United Carbon Products Company. To make sure the sample stayed on the surface of the electrodes, the surface was sealed with high purity heptane. The samples were transferred to the electrodes by first dissolving the ash in 1/4 ml of a 35% doubly distilled nitric acid solution. Then a long stemmed eyedropper was used to transfer the solution to a pair of electrodes. After the samples were dried on the electrodes, they were ready for spectrographic analysis.

CHAPTER IV

SPECTROGRAPHIC ANALYSIS OF SAMPLES

An important consideration in a spectrographic analysis is the method of excitation of the sample. In an analysis for metallic elements the sample must be excited with enough energy to destroy the molecular structure, so that light characteristic to the individual atoms will be emitted. A high voltage, high current A.C. spark source was used for this investigation, patterned after that of Enns and Wolfe. (See Figures 6, 7 and 8). This source was specially designed and constructed for this analysis, so that it would be adequate in both precision and sensitivity.

Sensitivity is directly related to the maximum current density of the discharge. The maximum current density (J) is related to the breakdown voltage (V) the capacitance (C) and the inductance (L) as follows:

$$J = V \sqrt{\frac{C}{L}}$$

A high capacitance and low inductance therefore favor sensitivity. To obtain high sensitivity in the source constructed, a large (0.75 µf) capacitor was used and a small (160 µh) inductor was used.

It is possible to supply more energy to the sample

SPARK SOURCE

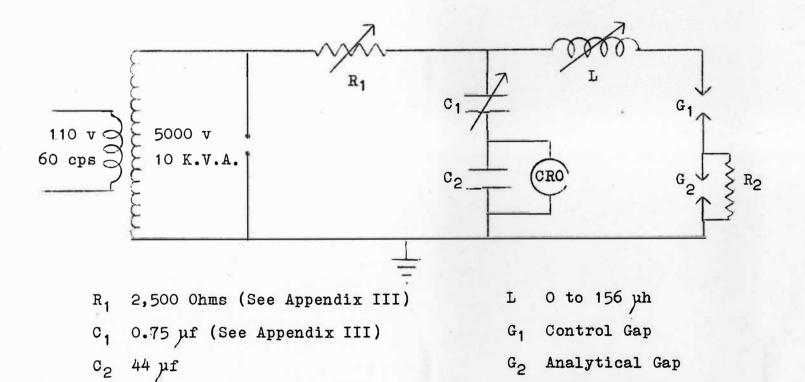


FIGURE 6

5 Megohms



FIGURE 7

This picture shows most of the components of the spark source diagramed in figure 6. The resistor (R_1) is partially hidden behind the transformer at the right. The inductance (L) is in the left foreground. The capacitors are partially hidden behind the inductor.



FIGURE 8

This shows the discharge compartment of the spark source. The electrode holders are in the middle of the compartment, a discharge between the electrodes may be observed in the center of the picture. The safety switch, in the lower right corner of the compartment, turns the power off when the door is open.

by having more breakdowns-per-half-cycle of the 60 cycle voltage wave. Four breakdowns-per-half-cycle or approximately one breakdown every 1/500th second was selected to be an effective value. A resistance value of 2500 ohms was used in the charging circuit to provide an RC time constant of 1/500th second.

A high precision was achieved by providing the sample with a constant excitation energy. A control gap between two tungsten electrodes was used to achieve a constant energy supply. A blast of air blown through this gap removed the ionized air and thus kept the breakdown voltage constant. The magnesium content of 3 identical 100 mg hair samples was reproduced with an average deviation of \pm 0.3 log ratio units using 10 second exposures.

A Cornu medium dispersion quartz spectrograph was used for the analysis. (See Figure 9). The light from the source fell upon the slit of the spectrograph. This slit was 30 microms wide and 6 mm high. Since this slit was positioned at the focal length of the collimating lens, parallel light passed from the lens to the prism where it was dispersed into its various wavelengths. The various wavelengths of light were then focused, by means of the camera lens, on an Eastman 33 photographic plate.

These blue sensitive, fine grain photographic plates were developed in D-19 developer for 2 1/2 minutes, then

OPTICS OF CORNU SPECTROGRAPH

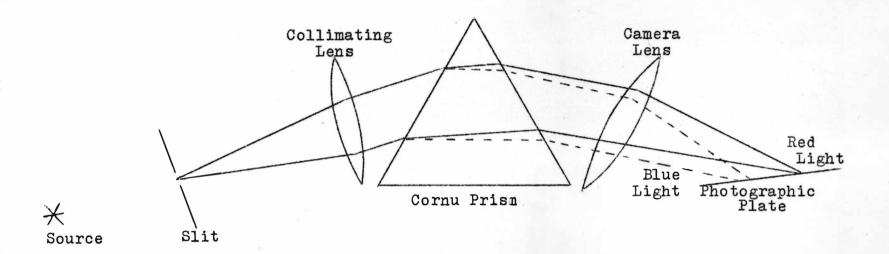


FIGURE 9

stopped in a 2% acetic acid solution for 1/2 minute before being fixed in Kodak rapid fixer for 2 1/2 minutes. During the full development precedure the glass developing trays were rocked mechanically at a uniform rate of 24 rocks per minute. This was done to insure a more uniform development by presenting fresh developer to the exposed portion of the plates at all times. This reduced the Eberhard Effect⁸ which occurs when the developer in the region of a dark spectral image is exhausted. The plates were then washed in running water for about 1/2 hour and rinsed in distilled water before being placed in a rack to air dry.

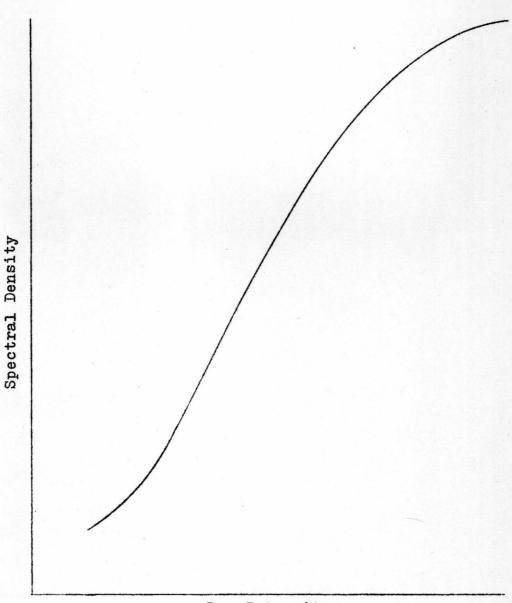
To determine how much of a certain element was present in the sample, the blackness of a line characteristic to this element, was measured on the photographic plate. Since the light from a given element is almost directly proportional to the quantity of the element present, the greater the concentration of a particular element, the darker its spectral image. An A.R.L. Dietert microphotometer was used to measure the spectral blackening. This instrument consists of a light cource focused on the spectrographic plate with a phototube to measure the amount of transmitted light getting through the photographic plate. This transmitted light energy is converted to electrical energy and displayed as a galvanometer read-

ing. A low reading means a small amount of transmitted light and thus a dark spectral image.

Previously it was stated that 0.003 mg of molybdenum was added to each sample as in internal control. The fact that the energy of the source must be kept constant was also mentioned. Actually it is impossible to eliminate all fluctuation of the source but these fluctuations should affect the internal standard in the same way that they affect the analytical elements, thus the ratio of the intensity of the two elements will not change. The internal control also minimizes error due to the loss of sample in transferring from the sample bottle to the electrodes. Since the solution is homogeneous any loss will not affect the ratio of the analytical element intensity to the molybdenum intensity.

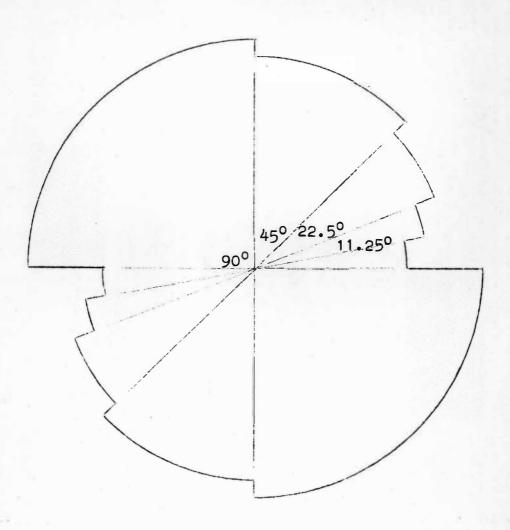
Each photographic plate has a different sensitivity to light, thus it is necessary to calibrate each plate. This calibration is made by means of an H and D curve (see Figure 10) which is the plot of spectral blackening (Microphotometer reading) against the log ratio of intensity. To make this calibration, a rotating step sector (see Figure 11) was placed in front of the slit during the exposure of the samples. This device exposed different portions of the slit in the ratio 1/8:1/4:1/2:1. Then the spectral blackening of each step was plotted against the

H AND D CURVE



Log Intensity

STEP SECTOR



SPECTRAL IMAGE OBTAINED USING A STEP SECTOR



FIGURE 11.

logarithm of the ratio of its exposure to 1. After this curve was made it was used in the opposite manner, to change the blackening of an analytical line and that of a molybdenum line to a log ratio value. The best results are obtained by choosing lines with intensities on the linear portion of the H and D curve. Using the molybdenum 2816A line for control, measurements were made on the following lines:

Fe 2599A; Mg 2783A; Mo 2816A; Si 2882A; Ca 3181A; Cu 3248A; Zn 3345A; Al 3944A; Ba 4554.

These spectral lines are identified in the hair spectra shown in Figure 12.

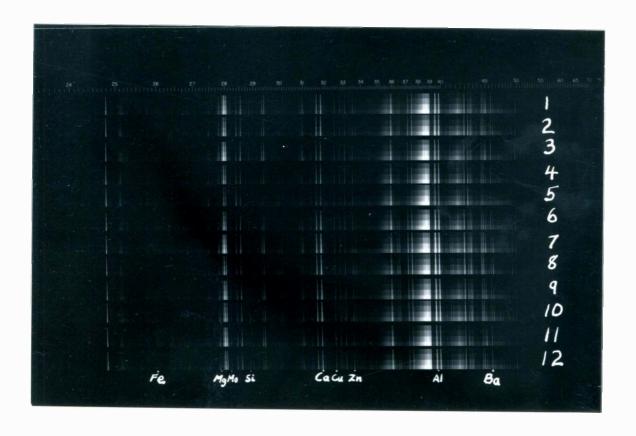


FIGURE 12

A contact print made from one of the hair analysis plates. The spectral lines used in this analysis are identified on this plate. Spectra 1, 2, 3, 5, 9, and 11 are from male coronary samples. Spectra 4, 6, and 12 are from male non-coronary. Spectra 7 is from a female coronary sample while 8 and 10 are from female non-coronary samples.

CHAPTER V

DATA AND RESULTS

The following charts, figure 13 to 20, illustrate the various metallic contents of the hair samples. Each element is presented on a separate page. For clarity the female and male samples are presented on separate but similar graphs.

The male samples are presented in eight groups, four coronary and four non-coronary with age divisions:

- 1. under 45
- 2. 45 through 54
- 3. 55 through 64
- 4. 65 and over

Since there were relatively few female samples, they were divided into only four groups:

- 1. coronary under 60
- 2. non-coronary under 60
- 3. coronary over 60
- 4. non-coronary over 60

The average metallic content of each group is plotted against the average age. A vertical line is used to represent the magnitude of the standard deviation.

The standard deviation (6) was calculated using the

formula:

$$\delta = \underbrace{\frac{\sum (\Delta X)^2}{\sum n - 1}}$$

Where X is the difference between the log ratio of each element in the group and the average log ratio for the group and n is the number of samples in the groups.

Figure 13 shows that the average magnesium content of the male coronary samples is definitely higher than that of the male non-coronary samples. This appears to be in opposition to the correlation between coronary disease and the magnesium content of water supplies as presented in Figure 1. These findings, however, are supported by the literature. Hellerstein et. al. 9 found that when the magnesium intake of rats with an atherosclerotic condition was increased, their condition improved. Other researchers 10,11 have observed that the concentration of magnesium increased with progressing age and atherosclerosis in the human aorta.

There is a difference between the magnesium levels of the female coronary and female non-coronary for the older group. This difference might have been more significant if it had been possible to analyze a larger number of samples.

The charts for zinc and calcium (see Figures 14 and 15) reveal a slight difference between the coronary and

non-coronary male samples. However, this difference is not nearly as significant as the difference observed in the magnesium.

For copper, (see Figure 16) there is no significant difference between the coronary and non-coronary groups, but the average copper content of the female samples is higher than the male samples. The average content of the female samples was found to be at least slightly higher than the male samples for all the elements studied.

Figure 21 shows that the average ash weight of the female samples is higher than that of the male samples. This fact tends to agree with the observation that the metallic content of the female samples was higher than the male samples for all of the elements tested. Another important fact to note is that there is very little difference between the ash weight of the coronary and the non-coronary group. Therefore any differences in the content of a particular element would represent a true unbalance in the metallic content of the hair.

This study revealed no significant differences between the coronary and the non-coronary groups for the elements copper, iron, silicon, aluminum and barium. However, it is quite possible that one or more of these elements might be correlated with some other disease or

common characteristic of the sample donors. This possibility is illustrated in Figures 22 and 23. Each of the four samples from people with myasthenia gravis are substantially below average in barium content. They were also a little below the average in the other elements analyzed. So little is known about this disease that any information provided will be helpful.

Figure 22 also shows that the girl with a severe case of falling hair has a slightly less than average amount of barium in her hair.

In Figure 24 the average copper content of the men with brown hair appears to be higher than that of either the blondes or those with black hair. No correlation with color was observed in the other elements. Also no correlation was observed between the various metallic contents and per cent of gray in the hair samples.

MAGNESIUM

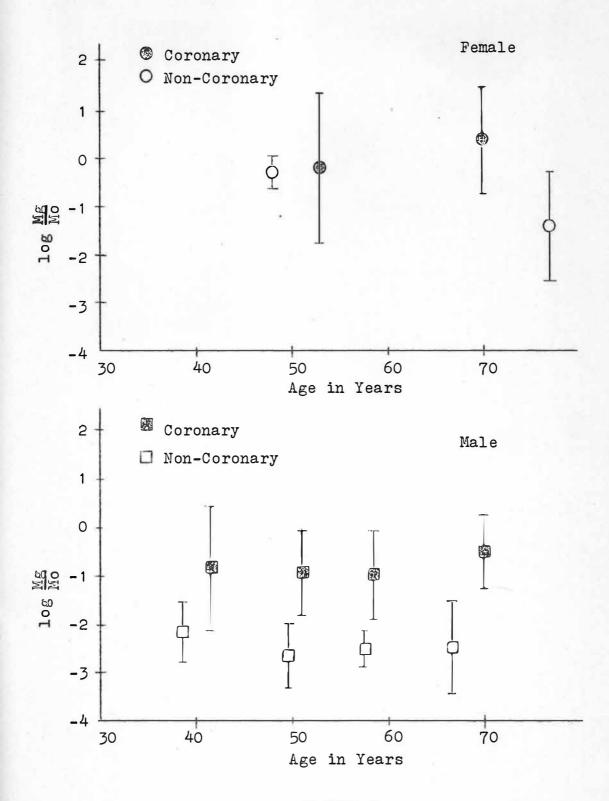
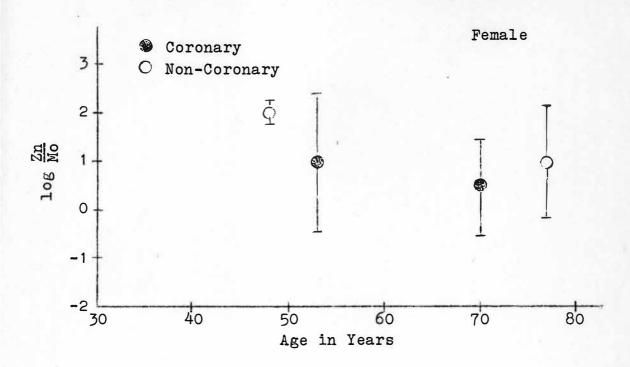
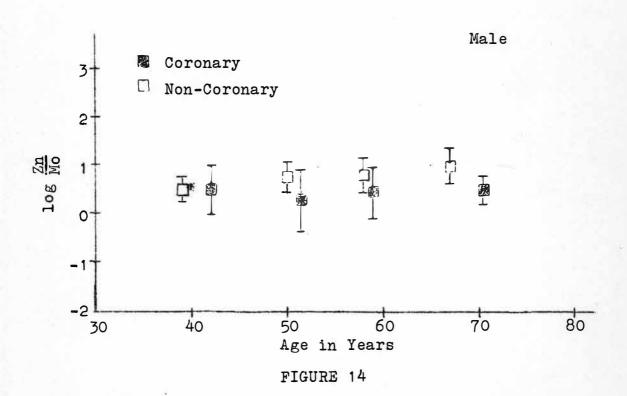
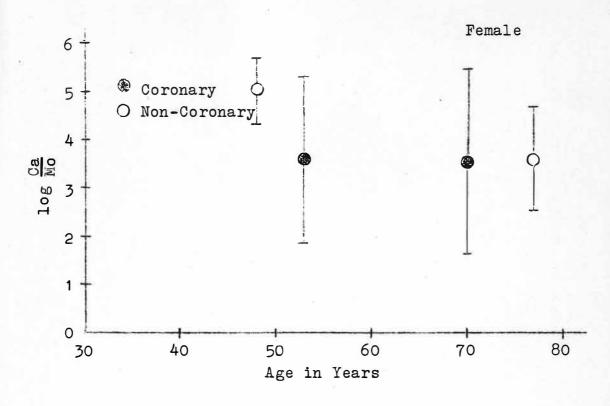


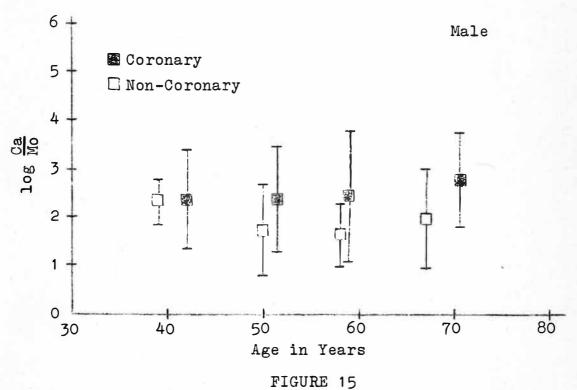
FIGURE 13



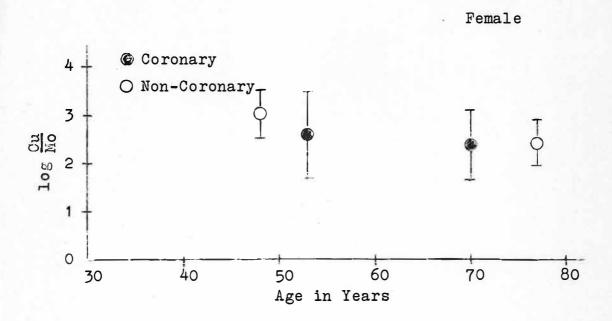


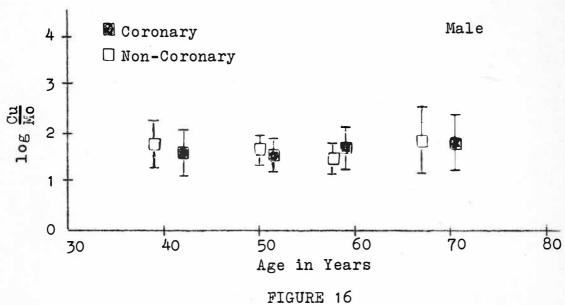
CALCIUM



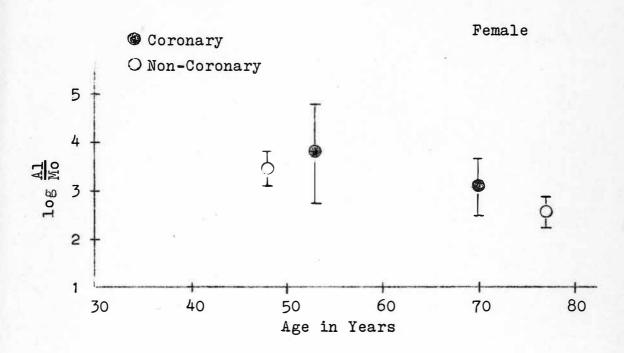


COPPER





ALUMINUM



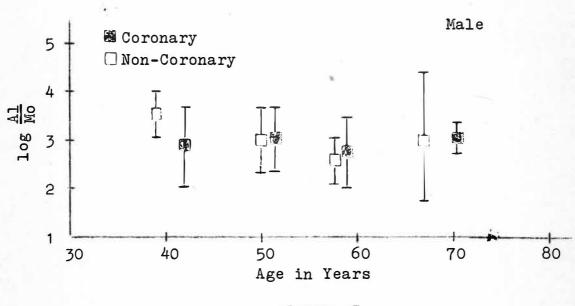
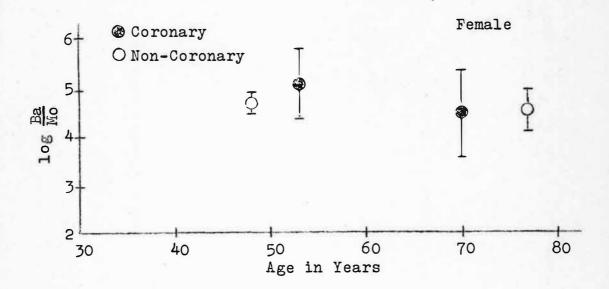


FIGURE 17

BARIUM



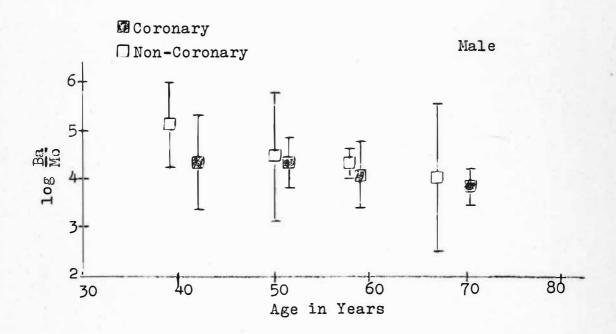
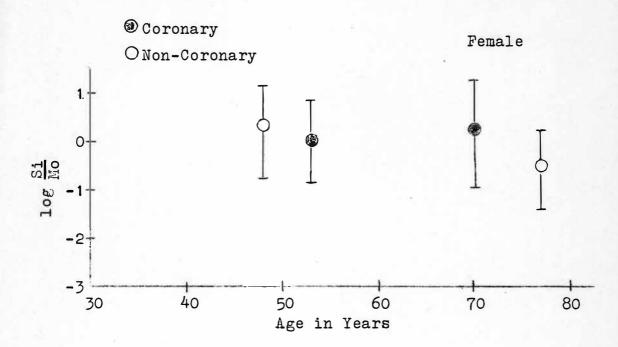


FIGURE 18

SILICON



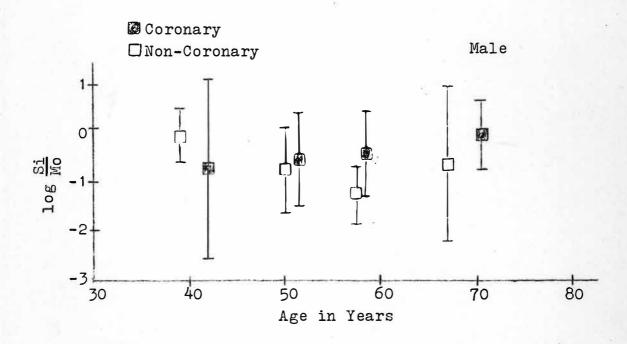


FIGURE 19

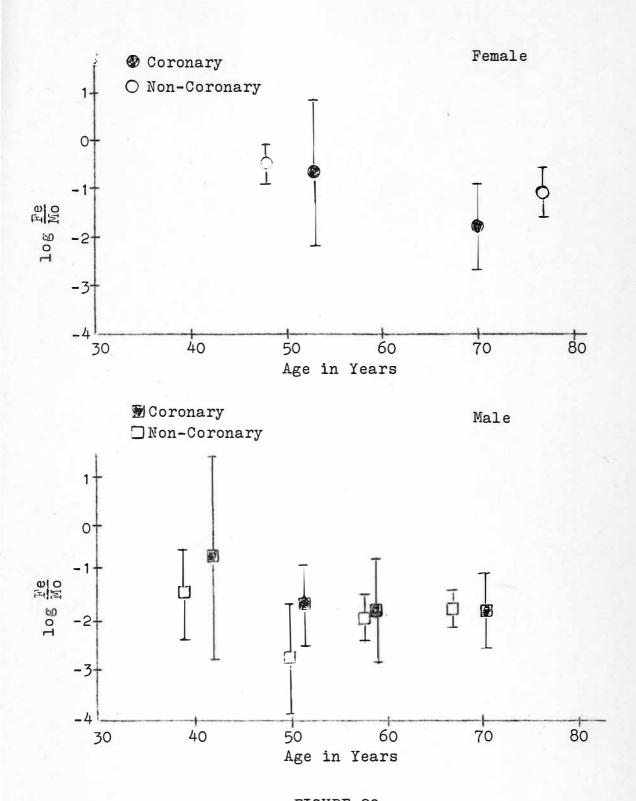
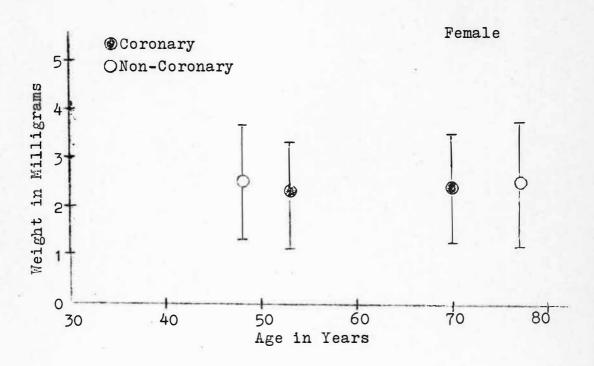


FIGURE 20

ASH WEIGHT



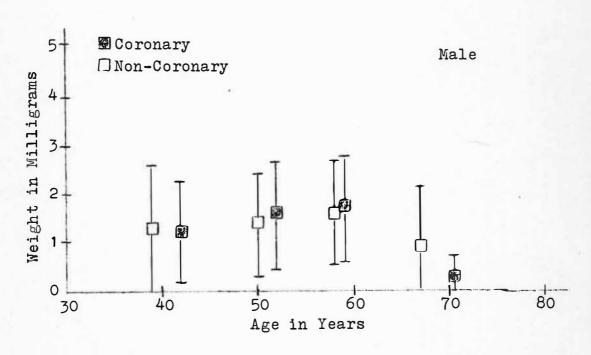
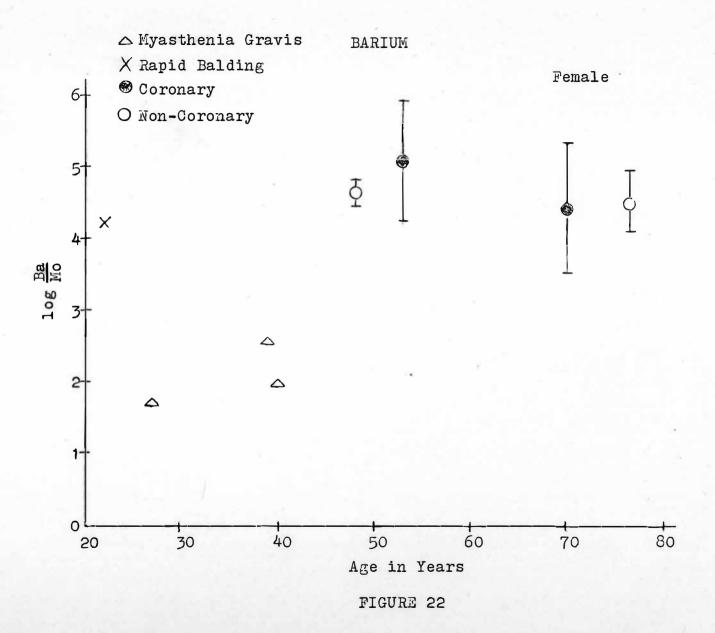
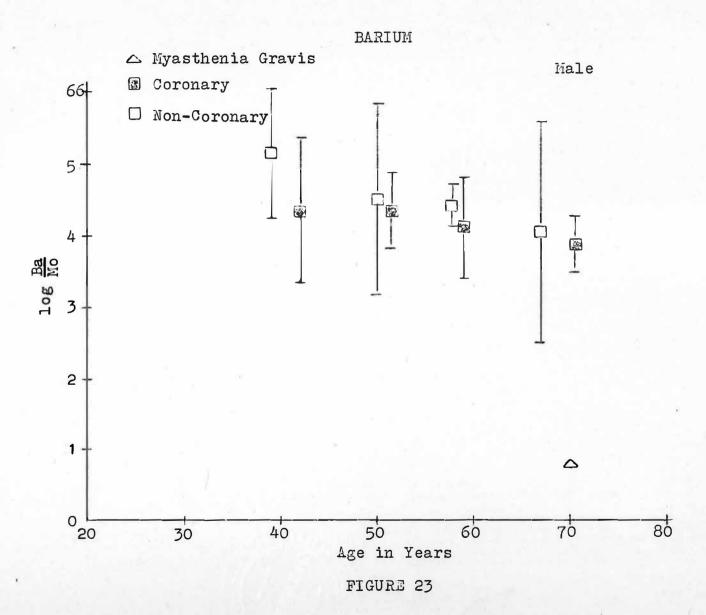


FIGURE 21





CALCIUM CONTENT OF MALE HAIR

VS

HAIR COLOR

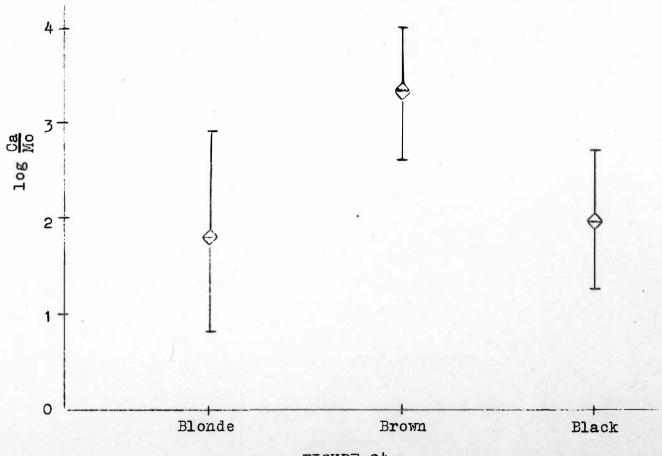


FIGURE 24

CONCLUSION

This report has outlined in detail the procedure for preparing and anlyzing hair samples for metallic elements.

The main objective of this thesis was to investigate a possible correlation between the metallic content of human scalp hair and coronary heart disease. Such a correlation was predicted to exist in particular for the magnesium and this investigation has established that the predicted correlation does exist. In fact, the analysis demonstrated that the magnesium content of male coronary samples was approximately double that of non-coronary males.

Information was also presented concerning the relative content of the elements magnesium, iron, silicon, aluminum, zinc, calcium, copper and barium for female, male, coronary and non-coronary hair samples.

Since standard curves were not available, the information was presented in log ratio units which are nearly proportional to the logarithm of the concentration. The approximate concentration of most of the elements studied appears in the referenced literature. 12

This report also showed that investigations of human hair with respect to other diseases could contribute additional information for the advancement of medical science.

KALAMAZOO, MICHIGAN

APPENDIX I

DOCTOR'S LETTER

I am writing to you concerning a study of possible correlations between the metal content of human hair and coronary disease. The attached page will furnish additional information concerning the project. Mr. Lamar Bullock is participating in this research for his Master's thesis.

We need help in collecting coronary patient hair samples. We would appreciate very much your cooperation in the collection of these samples. It is proposed that the collections would be made in the following manner.

- 1. We supply you with letters to patients (similar to the one enclosed) soliciting their cooperation.
- 2. Your secretary would address envelopes to your patients of known coronary ailments. Your secretary would then seal the letters and mail them with our postage. (This will protect the identity of the patients.)
- 3. Your patient would then make his (or her) own decision as to his willingness to send a sample of his hair to us in the stamped envelope provided.

If you would be willing to assist us in the collection of samples in a manner similar to that proposed we would appreciate your return of the enclosed post card. We will submit a report of our findings to you by July 1, 1962.

Thank you for your interest in our work.

Sincerely,

Stanley K. Derby Associate Professor of Physics

SKD/hh

WESTERN MICHIGAN UNIVERSITY

KALAMAZOO, MICHIGAN

APPENDIX II PATIENT'S LETTER

Dear Sir:

Your doctor has consented to forward this letter to you for your consideration.

As the enclosure indicates, we have been engaged for some time in the determination of the metals which exist in human hair. We have encountered some evidence of a possible correlation between the quantity of magnesium in human hair and heart disease. In order to carry out further investigations of this topic we need samples of hair from people with coronary ailments.

We would appreciate it very much if you would consider sending us a sample of your hair. This sample should be at least as large as a 3/4 inch marble when compressed into a ball.

We ask that you then place the hair sample in the envelope provided and mark your age and sex on the outside. Neither your name nor address need be included unless you wish to receive a free written report of our progress on July 1, 1962. No names will be used in any report of our work.

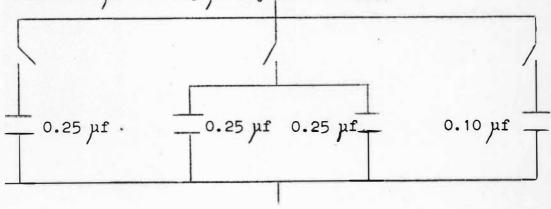
Thank you for your consideration of our request.

Sincerely,

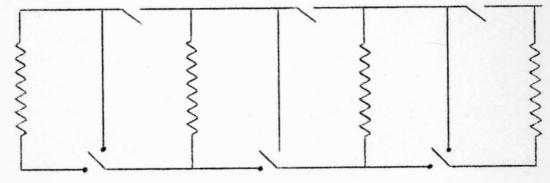
Stanley K. Derby Associate Professor of Physics

SKD/hh

Details of construction of capacitor C_1 of Figure 6. By choosing various switch positions capacitance values from 0.10 μf to 0.85 μf may be obtained.



Details of construction of resistor R_1 of Figure 6. Each resistor is a 200 watt 5000 ohm resistor. By choosing various switch positions resistance values from 1.25 K to 20 K may be obtained.



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