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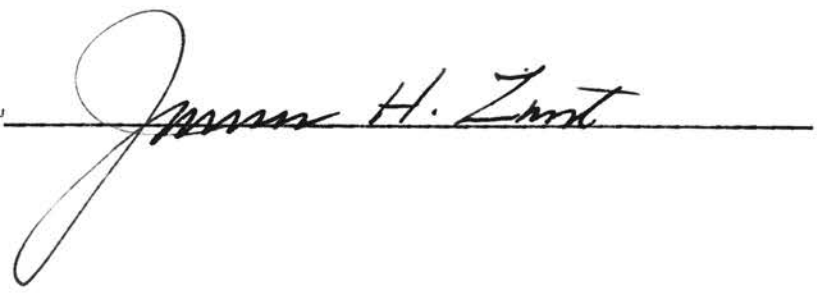
Scope of Study: High school laboratory manuals of chemistry were examined for biochemistry content. High school chemistry teachers were also consulted about the biochemistry phase of secondary school laboratory work. The major portion of the report is the collection and presentation of the experimental projects in biochemistry. The larger part of the information used in devising and adapting these projects is common to most biochemistry or chemistry text books. Some materials were obtained from other sources as noted.

Findings and Conclusions: Very few laboratory manuals for high school chemistry contained biochemistry experiments. Many high school teachers expressed a desire for more work of this nature but lacked a source from which to obtain it. This report provides a number of experimental projects in biochemistry which can be performed in most high school laboratories.

ADVISER'S APPROVAL 

EXPERIMENTAL PROJECTS IN BIOCHEMISTRY FOR HIGH SCHOOL LABORATORY WORK

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EXPERIMENTAL PROJECTS IN BIOCHEMISTRY

FOR HIGH SCHOOL LABORATORY WORK

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CHAPTER I

PURPOSE OF THE REPORT

After several years of teaching and consulting with other teachers of high school chemistry, a need for more adequate coverage of biochemistry and also for experiments of this nature became apparent. Especially is this so since the course is a terminal one for many people.

The fact that chemical and physical changes are fundamental aspects of any chemistry course is commonly accepted. However, their various relationships to living organisms is given very little attention in most high school courses. This seems to result from the fact that high school text books, in general, offer a very limited amount of material on the subject of biochemical relationships. The existing laboratory manuals appear to be even more limited, with some placing no emphasis at all on this subject.

The field of biochemistry is very broad and has been divided into many specialized branches. It is realized that on the secondary level only a few of the elementary phases can be profitably taught. However, there is a definite need for teaching high school students as much as possible about the processes we call living and growing. Health is one of the most important aspects of their lives.

In conferring with many high school chemistry teachers, most of them are questioned quite often, by students, about biological situations in which chemistry can be applied and, more important, understood. These teachers also receive many questions about current news items and reports in this field. Health, antibiotics, particular diseases, and many other subjects of this nature concern the populace directly. Articles of this nature many times contain technical terms and information which are beyond the scope of the average person. Because of this, misconceptions frequently arise. These lead to confusion and in some instances to the detriment of persons concerned. In many cases, students in high school can acquire a sufficient background to avoid this type of confusion. At the same time, some of the knowledge gained by these students is passed on to others, thus extending the results even more.

In addition to gaining basic knowledge about certain processes, consideration should be given to analyzing and evaluating scientific news items and magazine articles. These are very numerous and probably the ones pertaining to health are most widely read. Along this line, the following is an example. One report mentions the fact that the Bantu natives of South Africa have a very low incidence of heart disease and atherosclerosis.[1] It as noted that their

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[1] "Diet Secret of the Bantus," Science News Letter, Vol. 71, No. 11 (1956) p. 163.

diet consisted largely of cereal grains and very little fat. Also, the average level of cholesterol in the blood among these natives was very low when compared to that of a well fed person of this country. Does this mean we should immediately alter our diet in some radical manner? To some, this might appear to be the solution, since heart disease is so prevalent. In the instance cited, further study indicated other particular ailments of these natives which might have been caused by a lack of fat in the diet.

For class work along this line, as many pertinent articles as possible can be examined. Reference books and texts can also be used. Definite facts soon show up. There seems to be a relationship between cholesterol levels in the blood and fat intake. Also, cholesterol is associated with certain type heart ailments. However, even though fat ingestion is eliminated as nearly as possible, large amounts of cholesterol are synthesized from other substances in the body. Other facts apparently relate age and obesity to blood cholesterol level.[2] Finally, it appears that there is not sufficient information at present to draw any definite conclusions applicable in all cases. It can be pointed out that specialists are able to apply the various known facts to specific cases but, for the most part, we must wait and let science add more information to this particular subject.

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[2]William H. Peterson and Frank M. Strong, General Biochemistry. New York: Prentice-Hall, Inc., 1954, p. 95

Then we might possibly apply the known facts to the general public.

In this connection, it is realized that a teacher, not well informed, can disseminate false or confusing facts to students. This is inherent in any teaching situation. A good teacher is aware of his capacities and limitations and makes allowances for them. He is also aware that increasing his knowledge and background will improve his teaching ability. It is not expected that high school chemistry teachers should make biochemistry the major portion of the course. The writer does feel that, in the average situation, not enough emphasis is placed on this subject. That fact that plant and animal life is definitely related to chemistry is especially important to agricultural communities. The primary concern of many communities in this general area is the production of plant and animal products, thus knowing this relationship can be of benefit in an economic sense.

A question may arise concerning the addition of more laboratory work to the high school chemistry course. How is this new material to be included in an already crowded laboratory schedule? There may be other answers to this question, but the following general program has been used by the writer for several years. In order to gain laboratory time for more important things, there are a number of ordinary experiments which can easily be eliminated from the individual work. For instance, the experiments involving the preparation and properties of oxygen, for the most part, deal with facts and

information that can be obtained directly from the text. The material in this type experiment can be used by the teacher for demonstration purposes during the regular classroom coverage of the topic. More teacher demonstration can emphasize correct laboratory techniques and illustrate more vividly the points being discussed. The use of this type experimental material for demonstration requires little, if any, more class time than normally used. As students record observations and results in the manual, they will see an experienced teacher do much more than follow directions in a given experiment. Ways of making observations under adverse conditions, methods of getting precipitates when they seemingly will not form, adapting limited equipment to meet particular needs, many things of this nature will be presented which might not be encountered otherwise.

The problem of which and how many of the ordinary laboratory manual experiments should be utilized in this manner is for the individual teacher to decide. The biochemical experiments and projects included in this collection can be used as regular laboratory work or as outside projects or both. It is also possible that chemistry and biology classes might work together on some of the projects or experiments.

In order to properly understand and present the limited amount of material on biochemistry in the high school text books, a teacher, almost of necessity, must have at hand additional facts and information on this subject. There are good texts, both elementary and advanced, in the field

of biochemistry. These can be readily obtained and used to supplement subject matter when it is necessary. The problem of finding experiments for demonstrating particular biochemical processes and techniques is quite different. The average high school laboratory manual is not satisfactory and the college manuals are too advanced or require materials or equipment not usually available in a high school laboratory. With the realization that improvement can probably be made on them, the following experiments have been adapted and devised to provide some laboratory work for this important phase of high school chemistry.

CHAPTER II

EXPERIMENTAL PROJECT 1: Qualitative Determination of Starch and Glucose in Green Plants.

INTRODUCTION: During the process called photosynthesis the compounds carbonddioxide and water are converted into products which plants utilize for growth. Two substances which are often stored for later use are starch and glucose. The purpose of this experiment is to show the presence of these compounds in plant leaves. Many types of leaves can be used but some give a positive reaction more easily than others. Potted geranium plants are easy to care for and use. For this reason geranium leaves will be mentioned in other later experiments. The results will be better if the leaves have been in bright sunlight for at least one hour preceeding the experiment.

MATERIALS: Geranium leaves, ethyl alcohol, iodine solution, Benedict's solution.

PROCEDURE: For the determination of starch place about 50 ml of ethyl alcohol in an evaporating dish and heat over a water bath to boiling. Place a fresh leaf in the alcohol and continue boiling a few minutes. This dissolves most of the chlorophyll thus removing a greater part of the color from the leaf. Remove the leaf and quickly rinse in hot water. Place the leaf on a watch glass and add several drops of io-

dine solution. Starch is indicated by the characteristic dark blue color.

For the glucose determination place a finely chopped leaf in a 100 ml beaker with about 50 ml of water. Boil for several minutes and pour off about 15 ml of the solution into a test tube. To this add about 5 ml of Benedict's solution and bring to a boil. A reddish orange or yellow precipitate indicates glucose. Where small amounts of glucose are present the solution may have to stand awhile before the precipitate becomes evident.

EXPERIMENTAL PROJECT 2: The Effect of Carbon Dioxide in Photosynthesis.

INTRODUCTION: The actual importance of carbon dioxide in the synthesis of carbohydrates by plants will be shown in this experiment. The relative amounts of starch contained in the leaves of plants placed under certain conditions will be used for drawing conclusions.

MATERIALS: Ethyl alcohol, 10% sodium or potassium hydroxide solution, iodine solution, vaseline, and several plants which have been kept in the dark for two days. One should be small enough to put under a battery or bell jar.

PROCEDURE: Arrange a battery jar in bright sunlight. Place in it a small beaker containing potassium or sodium hydroxide solution. Cover the jar tightly for ten minutes; then carefully slide the cover aside and quickly put a small plant which has been kept in darkness inside the jar. Immediately recover the jar and leave it in bright sunlight for several hours. The purpose of the hydroxide solution is to absorb carbon dioxide. This set up will not eliminate all carbon dioxide but it will greatly reduce the content in the jar. This should be apparent when the starch test is made.

Another part of this experiment consists of putting a thin coating of vaseline on several leaves of a plant which has been kept in darkness. This will allow the passage of light but not carbon dioxide. Place the plant in bright sunlight.

After several hours test a few of the coated and also

some uncoated leaves from the plant for starch content. Perform the starch test using leaves from the plant in the battery jar. By comparing the results of these tests some diffences will be noted which should lead to definite conclusions about plants and their environment.

EXPERIMENTAL PROJECT 3: Photosynthesis and Chlorophyll.

INTRODUCTION: The term photosynthesis, in general, means synthesis or building by the use of light. As applied to chlorophyll containing plants it usually means synthesizing carbohydrates. Again, the relative amounts of starch present in the leaves will be used for comparison. In this case, the effect of light and darkness on the photosynthetic process will be compared. Also, by using a plant having varigated leaves, an estimation of the activity in the pigmented and non-pigmented parts of the leaves can be made.

MATERIALS: Geranium leaves, ethyl alcohol, iodine solution. Prior to the experiment, one geranium plant should be kept in darkness for two days. Another should be exposed to bright sunlight for several hours along with a plant having varigated leaves.

PROCEDURE: The test for starch in green leaves previously given will be repeated here. First, perform the test on a leaf from the plant kept in darkness. Then use a leaf from the plant exposed to light. Add the same number of drops of iodine solution to each and a total of not more than three or four drops. The difference in intensity of color gives a rough, quantitative indication of starch content.

Perform the same test using a varigated leaf. In this test, after the iodine solution has been on a few seconds, it may help to blot the leaf surface with absorbent paper such as filter paper. A definite contrast should be observed.

EXPERIMENTAL PROJECT 4: Plant Production of Oxygen.

INTRODUCTION: During photosynthesis in plants, carbon dioxide is taken from the air and utilized. At the same time, oxygen is being given off. The purpose of this experiment is to demonstrate that oxygen is produced.

MATERIALS: Water weeds or plants, wood splints, calibrated test tube. Any test tube with linear markings along its length will serve.

PROCEDURE: Place some water weed in the bottom of a large beaker or battery jar and fill about 7/8 full with water. Invert a short stemmed glass funnel over the plant. Fill the calibrated test tube with water and invert it over the funnel stem. In diffuse light observe the rate at which the gas collects in the test tube. Place the apparatus in direct sunlight and again observe the rate of gas evolution. When the gas has filled about 1/3 to 1/2 of the test tube, test it with a glowing splint.

EXPERIMENTAL PROJECT 5: Catalase Enzyme Reaction.

INTRODUCTION: The catalases are enzymes for which the primary function is not definitely known. They occur in the cells of practically all plants and animals, especially the higher forms. It is known that hydrogen peroxide, which is toxic, is produced in living systems. Apparently, one of the purposes of this enzyme is to destroy the hydrogen peroxide. This it does by decomposing it into water and oxygen. However, it is also known that, in the presence of ethyl alcohol, these enzymes catalyze its reaction with hydrogen peroxide to produce acetaldehyde and water. Similar reactions could occur in living tissues. In this experiment, plant material will be used with hydrogen peroxide and the amount of oxygen produced will be used to indicate the activity of the enzymes.

MATERIALS: Several types of fresh plant tissue, dead plant tissue, plant tissue not fresh, 3% hydrogen peroxide, calcium carbonate, wood splints.

PROCEDURE: Prepare a 10 gram sample of plant tissue by grinding it in a mortar with 1 gram of calcium carbonate. Use 20 ml of water and wash the sample into a small flask fitted with a one hole stopper and delivery tube. Have a pneumatic trough and an inverted calibrated test tube full of water available for collecting any gas that might be evolved. Add 10 ml of hydrogen peroxide to the flask and stopper it quickly. Observe the rate at which gas is generated. After the gas has been generating several minutes the test tube can be

removed and its contents tested with a glowing splint. Repeat the above procedure using dead material and some which seems to be in between. The same test performed on different types of fresh vegetation will give different results.

An interesting variation can be used in this experiment. Different parts of a given plant such as the roots, stems, and leaves can be compared with respect to the amount of activity in each. Also the viability of seeds of a given kind but from a different source can be compared by observing the amount of catalase activity.

EXPERIMENTAL PROJECT 6: Soil Organisms and the Production of Antibiotics.[1]

INTRODUCTION: Penicillin, one of the first antibiotics, was isolated from a common soil mold. Since then, practically all ordinary antibiotics have been produced from soil organisms. In the study of antibiotics and their uses, the stability, toxicity, and activity in low concentrations should be kept in mind. In this experiment some antibiotic substances usually develop during the incubation period. These can then be further studied. A rich garden soil or loam ordinarily contains organisms which are antibiotic. The developing of a given antibiotic for specific uses is a long and exacting process and only a few ever make the production line. This experiment could well be made a joint project with a biology class since many biological supplies are used.

MATERIALS: Sterile Petri plates, nutrient agar, nutrient broth, garden soil, inoculating loops and needles. Several common test organisms which are often cultured in biology classes are needed. Any of the following will do. Sarcina lutea, Escherichia coli, Micrococcus pyrogenes, Bacillus cerus, Serratia.

PROCEDURE: Mix thoroughly an arbitrary amount of rich garden soil with tap water and allow the mixture to settle overnight. The next day add one ml of the slightly turbid supernatant liquid to each of several sterile Petri dishes. Pour

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[1]This project was prepared from information furnished by the Department of Bacteriology at Kansas University.

melted and cooled (to 45° C) nutrient agar into each dish and mix well by rotating. Incubate the plates for 48 hours at room temperature.

After incubation, examine the plates macroscopically for evidence of colonies with clear zones around them. This indicates antibiotic action. There will usually be several colonies per plate which illustrate this phenomenon.

Using a sterile inoculating needle, pick several antagonistic colonies from each plate and place each organism in the center of a separate nutrient agar plate. Incubate the plates for two days, then streak in radial lines from the center where the antagonist is located each of the various test organisms. Allow the plates to incubate overnight and determine the extent to which each test organism is inhibited by the various antagonists.

EXPERIMENTAL PROJECT 7: Separation of Plant Pigments by Paper Chromatography.

INTRODUCTION: Chromatography is a method primarily used for separating mixtures of materials into their components. The exact mode of action is not definitely known. However, it seems that differential solubility and adsorption play an important part along with ion exchange reactions.

Many mixtures of closely related compounds are found in living tissue. For some purposes, chromatographic methods are best for separating and analyzing them.

There are several types of chromatography but probably the method using paper for the adsorbing medium is the most simple to use, at least for qualitative work. For paper chromatography, the materials to be separated must be in solution. In the method of this experiment a spot of the solution is placed on filter paper and allowed to dry. The separation is then effected by allowing another solvent to pass through or over the spot. This is done in a closed atmosphere saturated with the solvent and free from outside effects, such as drying and contamination.

MATERIALS: Methyl alcohol, ether, strips of filter paper 2 or 3 cm wide and 25 to 30 cm long, green leaves, or in the fall colored leaves can be used and compared.

PROCEDURE: Grind several green leaves in a mortar. The addition of fine sand may help in this process. Add 5 ml of water and 5 ml of methyl alcohol to the leaf pulp and stir thoroughly. Filter this and discard the residue. On

a strip of filter paper place a drop of the filtrate about 3 cm fromone end and allow it to dry completely. A hydrometer jar can be used for developing the chromatogram. Place ether to a depth of about 2 cm in the bottom of the jar. Suspend the paper strip so that the spot to be developed is about 2 cm above the surface of the ether. The paper will dip into the ether about 1 cm. Capillary action will draw the ether up the strip. The time required for the ascension will vary with conditions. When the solvent has risen 20 to 25 cm there should be a definite separation of the pigments. A greenish spot or band consisting of two types of chlorophyll should be nearest the bottom. Next above it there should be a yellow region for xanthophyll and then a reddish one for carotene.

There are many experiments and projects dealing with paper chromatography which are simple, informative, and interesting. The book by Cramer is a good reference source.[2]

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[2]Cramer, Friedrich, Paper Chromatography 2nd Edition, New York: Macmillan, 1954.

EXPERIMENTAL PROJECT 8: Glycerol Production by Fermentation.

INTRODUCTION: Under ordinary conditions, alcohol is produced by microbial fermentation involving sugar and yeast. If however, the pH of the medium is kept at about 8.5, the action of the yeast changes so that glycerol is a major product. Another method for increasing the amount of glycerol produced by fermentation is the addition of sulfites to the medium. The latter procedure will be used in this experiment.

Glycerol is an important compound used in many industries. It is a base for many pharmaceuticals; it is used in confectionary manufacture and in the production of explosives. The chief source of glycerol is animal fats.

MATERIALS: Yeast (dry or cake), sugar, sodium sulfite or sodium bisulfite, lime.

PROCEDURE: Crush one cake or package of yeast with sufficient water to make a paste. Place about 125 grams of sugar in a liter flask and fill to about 7/8 full with water. Add the yeast paste, shake well, and let stand at room temperature for about one hour. Then add approximately 45 grams of sodium sulfite, stopper tightly, and allow to stand about one week at room temperature. Agitate the contents whenever possible during this time. After fermentation, add about 25 grams of lime to the flask and shake. Allow contents to settle for 5 minutes and then filter. Use fractional distillation to separate the glycerol from the filtrate. Examine the product and compare it to a stock sample of glycerol.

EXPERIMENTAL PROJECT 9: The Nature of the Non-protein Nitrogen of Blood Serum.[3]

INTRODUCTION: This project is not especially difficult but it does require care and patience. It also requires a knowledge of two dimensional paper chromatography. Directions for several methods of paper chromatography are given in the manual by R. J. Bloch.[4]

It is generally considered that the body pool of nitrogen is contained in the blood serum, but the nature of the pool, if actually present at this site, has not been adequately demonstrated. It has been shown that free amino acids are present in blood serum, but in amounts too small to satisfy the continuing requirement for exchangeable nitrogen. Other forms of a circulating nitrogen are represented by such compounds as urea, uric acid, and creatinine, but in relatively small quantities, and since these substances are excretory forms of nitrogen, they cannot be a part of the exchangeable nitrogen pool. It has therefore been suggested, but not proved, that the major portion of the nitrogen pool may consist of circulating polypeptides in the blood serum.

If this latter suggestion is correct, it should not be difficult to obtain confirmatory experimental evidence. A direct and fairly simple approach could be based on differential analysis of protein-free filtrates of blood serum

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[3]This project was suggested by Dr. H. W. Barrett of the Department of Biochemistry at the University of Kansas.

[4]R. J. Bloch, Paper Chromatography. Academic Press Inc. New York, 1952.

before and after hydrolysis of the filtrates. An experiment based on this approach is outlined below.

MATERIALS: Ninhydrin, 10% trichloracetic acid, Amberlite resin IR4B, Amberlite resin IRC-50 (H), barium hydroxide, 6 N sulfuric acid, amino acid kit, blood serum. It is usually possible to obtain pint quantities of outdated blood serum from hospitals or clinics, and this material can be used as obtained. Alternatively, animal serum can be used after allowing the whole blood to clot, centrifuging, and drawing off the clear supernatant serum.

PROCEDURE: To obtain a protein-free filtrate use about 15 to 25 ml of the blood serum. Add slowly, with stirring, a solution of 10% trichloracetic acid until no further precipitate forms. Allow to stand for 30 minutes, then filter through quantitative filter paper. Wash the precipitate by suspending it in a small amount of water, stirring, then filtering. Discard the precipitate, and combine filtrates.

For desalting the filtrate, place it in a convenient sized flask; add a portion of Amberlite resin IR4B and shake well. Decant the liquid and wash the resin well with three portions of distilled water. Combine the washings with the original decanted liquid. Repeat this procedure with Amberlite resin IRC-50 (H). The resins should be thoroughly washed with distilled water before desalting. Exact quantities cannot be predicted in this step. It is required to remove strong inorganic ions with organic ions being left in the solution. A few preliminary tests will indicate the amount

of resins to use.

Paper chromatography will be used for separating the amino acids and peptides. Concentrate the final liquid to a small volume, short of appearance of a precipitate. This can be done by placing the liquid in an evaporating dish over a large beaker of warm water, and blowing a stream of dry air over the surface. A portion of the concentrated liquid, about 0.03 to 0.05 ml, is placed on a sheet of filter paper and submitted to two dimensional chromatography. Finally, the amino acids and peptides are located by spraying the chromatogram with ninhydrin reagent. For this chromatography, sheets of Whatman #1 filter paper about 24 cm square are recommended. The chromatography can be carried out in any large glass jar with a close-fitting cover.

The remaining liquid is hydrolyzed by adding an excess of barium hydroxide and heating on a steam bath for at least 4 hours. Keep the liquid level constant. At the end of this period, the barium ion is removed as barium sulfate by adding an equivalent quantity of sulfuric acid to the solution at room temperature and filtering. The filtrate is chromatographed as before, and the amino acids located with ninhydrin. A comparison of the two sheets will determine whether additional amino acids were released from peptides in the barium hydroxide hydrolysis.

Mixtures of pure amino acids should be made in microgram quantities from an amino acid kit. These can then be chromatographed in the same way to provide standard chromatograms.

EXPERIMENTAL PROJECT 10: Some Effects of Poisons on the Respiration of Plants.[5]

INTRODUCTION: Plants and animals obtain all the energy needed for growth and maintenance by oxidation of foods. During this oxidation, carbon dioxide is produced and oxygen is used. A convenient method of measuring the rate of oxidation is by placing the tissue in question in a vessel whose volume can be accurately measured. If provision is made to absorb the carbon dioxide as it is produced, then as oxygen in the air in the vessel is used up, the volume and pressure inside should decrease, and the change be measurable.

Plant tissues differ from animal tissues in that they frequently contain large amounts of stored food, usually carbohydrates. An excellent example is found in seeds, such as oats. When seeds sprout, respiration is very rapid and the tissue can be sustained for long periods of time on the carbohydrates stored in the seed.

Many chemical compounds are poisonous both to plants and animals because they inactivate certain enzymes involved in the oxidative reactions of respiration. When respiration stops, the tissue can no longer obtain energy, growth stops, and death occurs.

Some of these poisons are sodium cyanide, sodium azide, sodium sulfide, carbon monoxide, and sodium fluoride.

The procedures suggested here are designed to determine

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[5]This project was suggested by Dr. R. C. Mills of the Department of Biochemistry at the University of Kansas.

the effects of several compounds on the respiration of germinating oat seeds and to determine the concentration of each required to cause inhibition, if any.

MATERIALS: Oat seeds, 10% sodium or potassium hydroxide solution, 0.01 N solutions of potassium cyanide, sodium sulfide, and sodium azide. These solutions may be used during the same experiment to compare the effect of the different substances, or a single compound may be used in various concentrations for a comparison of effects. Other compounds and combinations may be used as desired.

PROCEDURE: Oat seeds are prepared by removing the hulls individually from the seeds and then soaking the seeds in water for 8 to 12 hours at room temperature. As soon as germination is obvious, the seeds may be used for respiration studies.

Respiration is followed in a respirometer.[6] Enough colored solution should be placed in the arms of the manometer to fill the tubes about half-way. Place 30 of the germinated seeds in a respirometer; add 4 ml of water to the seeds and then carefully place 0.5 ml of potassium hydroxide solution in the center vial. Insert the stopper with the manometer tube tightly. Take care not to lose any manometer fluid. Adjust the moveable glass plug until the manometer fluid is at the desired level. For measuring oxygen absorp-

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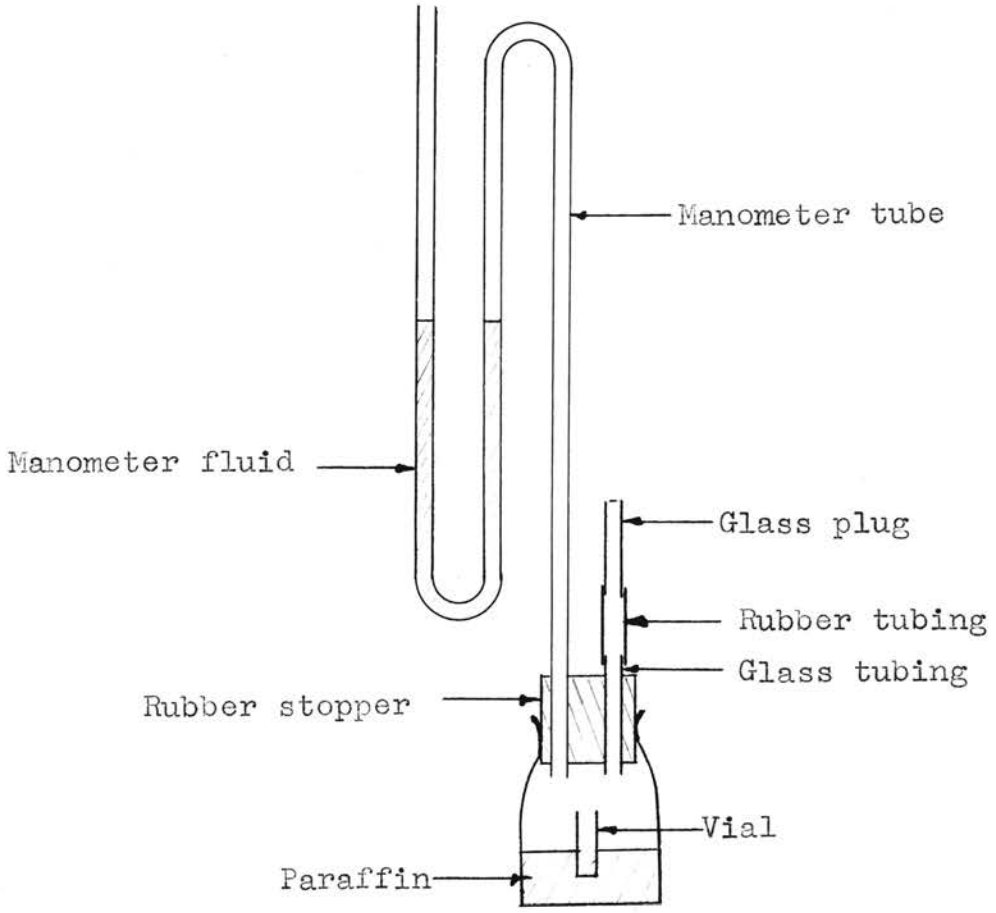
[6]Simple but serviceable respirometers, similar to the Warburg respirometer, may be made as shown in the drawing which follows this experiment.

tion, the fluid should be high in the open arm and low in the center arm of the manometer. This first respirometer will serve as a control. A second respirometer should be set up in exactly the same manner as the first except that no seeds are added. This will serve as a thermobarometer and measure changes in pressure and volume caused by temperature and atmospheric pressure changes. All experimental respirometers must be corrected for these changes at the time readings are made. Other respirometers are set up in the same way using the various solutions which are desired.

When all flasks are ready, they should be kept at room temperature. To facilitate handling they can be taped together in groups of 3 or 4. A large beaker or battery jar may be used as a water bath and will provide more uniform temperature. It is important that all respirometers be kept at the same temperature if a comparison of the results is to be worthwhile. Frequent mild agitation is desireable. Do not touch the respirometers with the hands. The readings of the various respirometers should be made as close to the same time as possible. The time between readings will vary with conditions. Measure the levels of the manometer fluids in millimeters. Express the results as millimeters of movment of fluid per minute or per 5 minutes, whichever is appropriate. The results should be graphed for comparisons.

This experiment can be done individually but it provides an excellent opportunity for teamwork.



This simple respirometer can be made from a two ounce wide mouth bottle equipped with a two hole rubber stopper. In one hole a small open manometer made of glass tubing is placed. In the other hole place a short piece of glass tubing. Close the rubber tubing with a snugly fitting but moveable glass plug. The bottom of the bottle should be filled with paraffin to reduce the effective volume to about 20 ml. A small glass vial is imbedded in the paraffin to contain the hydroxide solution. The vial should extend almost to the stopper to facilitate the addition of the hydroxide solution without spilling it into the rest of the bottle.

CHAPTER III

SUMMARY

The experiments included in this report are for supplemental use in the presentation of elementary biochemistry to high school students. The writer feels there is a need for placing a greater emphasis on this particular phase of chemistry. Since the existing high school laboratory manuals contain very few experiments of this type, this collection was made with the hope that it will partially alleviate the aforesaid condition.

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