PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

Founded December, 1908

VOLUME XVI

1962

Published jointly by the University of North Dakota and the North Dakota State University of Agriculture and Applied Science Sponsored also by the Jamestown College

July, 1962

GRAND FORKS, NORTH DAKOTA

TABLE OF CONTENTS

TABLE OF CONTENTS

SYNTHESIS OF THE 3-(o-METHOXYPHENYL)-ISOXAZOLES

Lynn A. Brandvold and F. H. Rathmann

Department of Chemical Technology

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

INTRODUCTION

The purpose of this work was to prepare and to study new compounds in the isoxazole series. The isoxazole ring compounds were prepared in the same manner as reported by Quilico and Fusco (1).

EXPERIMENTAL

Preparation of o-methoxybenzaldoxime

To 0.529 moles of H_2NOH-HCl and 0.264 moles of Na_2C0_3 dissolved in water, 0.44 moles of the o-methoxybenzaldehyde were added. The solution was heated and stirred for 1 hour and allowed to cool with continued agitation. White crystals of the oxime formed which were removed by filtering and were dried. The melting point was 90° —92° C. The reported value in the literature was 92° C (2).

Chlorination of o-methoxybenzaldoxime

Following the preceding, 0.20 moles of the oxime were dissolved in ether and 0.12 moles of chlorine gas were passed into the solution. The ether solution was exaporated leaving the chlorinated oxime which was used without purification.

Preparation of (3(o-methoxyphenyl)-5-methyl-4-isoxazole-carboxylic acid

To a flask cooled in ice, 0.108 moles of the chlorinated oxime in absolute alcohol and 100 ml of sodium acetoacetic ester were added. The solution was left to stand for 24 hours, and the precipitate of sodium chloride was removed by filtering. The alcohol was evaporated and ether was added. The solution was treated with dilute sodium hydroxide and the ether was distilled. Strong sodium hydroxide and water were added to the oily residue. The solution was boiled and 0.1N hydrochloric acid was added until precipitation occurred. The isoxazole acid was recrystallized from benzene yielding white crystals melting at 213°-214° C.

Preparation of 3(0 methoxyphenyl)-5-methyl-4-isoxazole acid anilide

Isoxazole acid was refluxed with thionyl chloride for 1½ hours in the ratio of .01 mole to 10 ml. The excess thionyl chloride was vaporized and removed by aspiration. The solution was divided into two equal parts; to one part was added 0.1 mole of aniline. A precipitate was immediately formed. The precipitate was filtered and

recrystallized from methanol yielding fine while crystals melting at 183°,-185° C.

Preparation of 3(o-methoxyphenyl)-5-methyl-4-isoxazone acid p-toluidide

The second part of the reaction mixture from above was reacted with 0.1 mole p-toluidine. A precipitate was immediately formed yielding light brown crystals which decomposed at 190°-194° C.

CONCLUSION

New compounds which have been prepared are 3(o-methoxyphenyl)-5-methyl-4-isoxazole carboxylic acid, 3(o-methoxyphenyl)-5-methyl-4-isoxazole carboxylic acid anilide and the 3(o-methoxyphenyl)-5-methyl-4-isoxazole carboxylic acid p-toluidide.

REFERENCES

1. Quilico, Adolfo, and Raffaello Fusco, "New Researches in the Isoxazole Group," Gazzetta Chimica Italiana, 67, 589-603, (1937), Chemical Abstracts 32:21174.
2. Beilstein, E II, Vol. 8, p. 43.
3. Rathmann, F. H., Thomas Farley and Duane Tangen, Proc. N. Dak. Acad. Sci. Vol. XIII, p. 61-65, 1959.
4. Rathmann, F.H., and Robert Saboe, "Studies on Isoxazoles," Student research report unpublished.

COLOR PREDICTION OF MACARONI PRODUCTS

Paul E. Cornelussen[1], L. D. Sibbitt, K. A. Gilles

Department of Cereal Technology

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

INTRODUCTION

Various methods are used to predict the color of macaroni, each method having its own characteristic degree of accuracy.

Perhaps the most simple method is the observation of the relative color of the dry semolina to be processed. This is not dependable because of the lack of consideration of the changes which the semolina undergoes during processing.

Next in complexity is the color test proposed by Pekar (1). Although originally used on flour samples it can be modified to allow comparison of processed semolina samples. The method uses a wetted and dried sample but there is no pressure treatment and the results are unreliable and inconsistent.

Another method proposed by Winston (2) calls for the packing of semolina to a uniform depth in a tray of standard size, followed by wetting and drying of the sample. Again there is very little pressure treatment and the results are inconsistent.

The method with which this report deals is the pressed disc method proposed by L. D. Sibbitt. This subjects the semolina to all the processes undergone by macaroni itself, save the process of extrusion and drying. Although the pressure treatment and the tedious drying process are not the same in macaroni production, there is a good correlation between the color of the macaroni and that of a pressed wet disc processed from the same semolina sample.

Operation of the MacBeth-Munsell Disc Colorimeter, Type 1 (4)

The color discs are interlaced in the order of black, white, yellow and red and fastened on the spindle in such a way that when the disc is spinning the direction of rotation will flatten the exposed edges. The light source, using a voltage stabilizer, provides a uniform, diffused illumination. The light intensity is checked periodically with the use of a photographic exposure meter.

The sample is positioned, the light source is activated and the color disc set in motion. The sample and the rotating disc are observed through an aperature in the viewing shield. Appropriate adjustments are made on the spinning disc until a perfect color match is achieved. This process of matching may require many adjustments, however, experience shortens the matching time considerably. When a perfect color match is obtained, the percentages of each color exposed on the disc are determined and recorded.

MATERIALS AND METHODS

Pressed Disc Method

Thirty grams of semolina are mixed with water at 30°C. for two minutes in a micro macaroni mixer changing direction of the mix every thirty seconds to insure uniform mixing (3). Water absorption of the semolina is very important and it may be necessary to predetermine this value. The dough should be slightly wetter than that normally used for macaroni processing. Figure 1. shows the color-water absorption relationship of a typical sample.

After mixing, the dough is kneaded four minutes in a micro kneader (3). It is then passed through smooth sheeting rolls, compressing it into a sheet of approximately 0.1" thickness. A disc 2¼ inches in diameter is cut from the sheet using a simulated cookie cutter, covered with a smooth metal foil, and fitted into a three piece plunger-sleeve apparatus. This disc is then subjected to a pressure of 2000 psi. for one minute in a Carver laboratory press. The pressure is released and the disc removed from the plunger base. The metal foil is peeled off and the color of the smooth surface is read using the colorimeter.

Color Scoring Methods

A point system of macaroni color ranking is used in the Cereal Technology Laboratory of N.D.S.U., which employs a scale of 1.0 to 10.0 in increments of 0.5. The best samples are graded as 10.0 and the poorest as 1.0. This method is strictly a visual classification and is dependent on the experience of a trained technician.

Nickerson Color Score (5)

An arbitrary single figure color score is derived from the following:

$$\text{Score} = \frac{\text{Hue x Chroma}}{\text{Brilliance}}$$

Example:

19% White (N9.6/)
13% Red (10R5.7/14.6)
30% Yellow (0.5GY9.2/10.8)
38% Black (N1.5/)

$$\text{Hue} = 30.5 - \left(\frac{(13 \times 5.7 \times 14.6)}{(13 \times 5.7 \times 14.6) + (30 \times 9.2 \times 10.8} \right) (30.5 - 10) = 25.04$$

$$\text{Brilliance} = \left(\frac{13(5.7^2 + 30(9.2)^2 + 38(1.5)^2 + 19(9.6)^2}{100} \right)^{\frac{1}{2}} = 6.93$$

$$\text{Chroma} = \frac{13 \times 14.6 + 30 \times 10.8}{100} = 5.14$$

$$\text{Score} = \frac{25.04 \times 5.14}{6.93} = 18.63$$

RESULTS

Forty-two samples of the 1960 durum crop were used in this study. These wheats were from the durum field plot variety trials grown at six locations in North Dakota. The samples were cleaned, milled into purified semolina and macaroni processed. The macaroni color score was determined using the procedure previously outlined.

A total of three methods for reporting color were used in this project. The Nickerson calculated single figure score and the percentage of "yellow" exposed on the MacBeth-Munsell color discs were applied on the semolina-water undried discs. These results were compared with the visual color score of the processed macaroni. A correlation coefficient of +0.8694 was obtained between macaroni color score and the Nickerson single figure score. When only the yellow section of the four color components was compared with the Nickerson score, a correlation coefficient of +0.9215 was obtained. When the visual macaroni color score was compared with the yellow percentage, the correlation coefficient was +0.8013. Figure 2 is a scatter diagram of the visual macaroni color score and the Nickerson

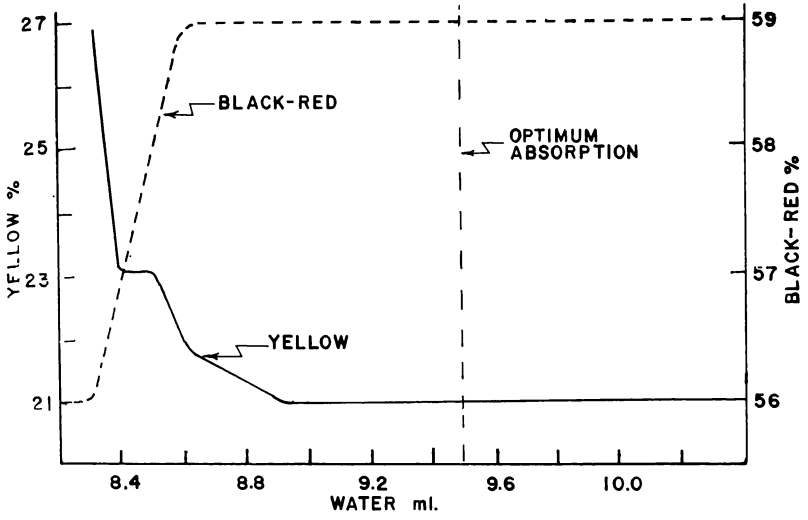


FIGURE 1—The effect of water addition on disc color.

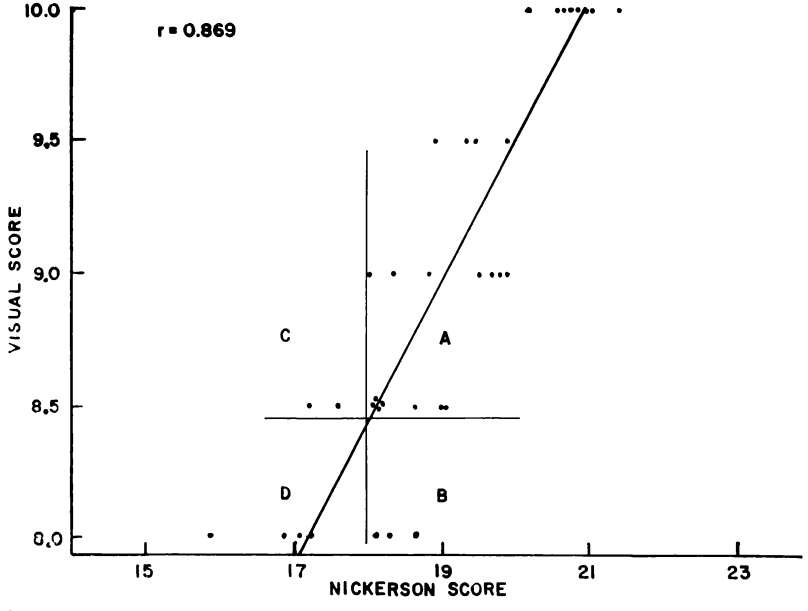


FIGURE 2—Comparison of visual color scores of macaroni and the calculated Nickerson scores of the pressed discs.

single figure calculated score. The graph indicates that the pressed disc method is 91.7% efficient in selecting color values of 8.5 and above, while the overal efficiency of predictions is 75.59%, (6).

DISCUSSION

The results indicate that the pressed disc method is quite accurate in predicting the color value of macaroni. If such a procedure were adopted in cereal laboratories, the need for the time consuming process of macaroni drying would be eliminated. The time required for this method, including duplicate runs, is about one seventh that of macaroni production. Therefore, the man-hours required would be greatly reduced, or the number of samples for analysis could be greatly increased.

In addition to the speed of analysis, another desirable factor is the elimination of some variables. In macaroni production, drying is one of the most critical factors. This variable has been removed in the pressed disc method. The color judgement is also improved. Color analysis in the colorimeter is not subject to varying intensities of illumination, outside glare or interference. Finally, experience in macaroni color grading is not necessary in order to match colors in the colorimeter.

LITERATURE CITED

1. Pekar (color test), Cereal Laboratory Methods 5:46 (1947).
2. Winston, James J., Personal Communication to Cereal Technology, (1961).
3. Harris, R. H., North Dakota Bimonthly Bulletin Vol. 9, No. 4, (1947).
4. Colorimetry, Cereal Laboratory Methods 5:175 (1947).
5. Nickerson, Dorothy, U.S.D.A. Technical Bulletin No. 154.
6. Banasik, O. J.; Myhre, David; Harris, R. H.; Brewers Digest, 31:65 (1956).

[1]Paul E. Corneliussen was a National Science Foundation Undergraduate Research Participant.

SARETT OXIDATION OF 1, 4-DIOLS, A SYNTHESIS OF GAMMA-LACTONES

Virgil I. Stenberg and Robert J. Perkins

Department of Chemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

A study is presented on the oxidation of a variety of 1, 4-diols using the Sarett procedure. A gamma-lactone is formed in all cases except where stereochemical orientation prohibits it.

The formation of the gamma-lactone is thought to proceed through an intermediate hemiacetal which is readily oxidized to the corresponding lactone. It is demonstrated that the formation of lactone requires vicinal hydroxymethyl groups to be essentially coplanar. Where the coplanarity is prohibited the expected Sarett product, a 1, 4-dialdehyde, is the major component.

The models chosen for study were **o** xylene-α, α-diol, **cis**-1, 4-butenediol, 1, 4-butanediol, **cis**-1, 2-bis-(hydroxymethyl)-cyclopentane, I, and **trans**-1,2-bis-(hydroxymethyl)-cyclopentane, Gamma-lac-tones were obtained in good yields except in the latter two cases. The latter two diols gave an identical 1,4-dialdehyde, the expected Sarett product for the **trans**-cyclopentanediol. Base-catalyzed isomerization of I presumably accurred on the intermediate aldehyde-alcohol.

ABNORMAL CHLORINE LOSS FROM OUTDOOR SWIMMING POOLS. REACTION OF CHLORINE WITH URINE, UREA AND URIC ACID

Larry Guilbert, Charles W. Fleetwood, and F. H. Rathmann

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

It has been noted that proper concentration of chlorine, used as a disinfecting agent in swimming pools, has become increasingly difficult to maintain. The problem is to determine what happens to the chlorine.

The chlorine usage may be ascribed to at least four different factors:

1. Loss of chlorine through the action of sunlight upon hypochlorous acid.

$$Cl_2 + H_2O \xrightarrow[\text{Radiant energy}]{} HCl + HOCl$$

$$HOCl \xrightarrow{\text{Radiant energy}} HCl + (O)$$

2. Loss of chlorine due to reaction with urine.
3. Leakage from the pool requiring fresh water addition.
4. Loss of chlorine in the outdoor pool through direct vaporization.

An increasing number of people used the Fargo pool through the years 1955 to 1957, with attendance leveling off after 1957. Increased use causes more influx of urine and perspiration. It has been observed that urine consumes chlorine in water solution.

Because some of the chlorine losses are interrelated, a complete picture of the pool environment was desired. For this purpose, the

pH, free chlorine, chloride, and nitrogen concentrations were determined during a 10-week period. In addition, various reports on attendance at the pool, chlorine consumption, hours of direct sunlight, and temperature were obtained from the Fargo Park Commission and the Weather Bureau at Hector Airport.

The results show that the largest consumer of chlorine from the pool was the sunlight reaction as the chlorine concentration at any one time of the day showed the effect of sunlight. This effect would not tend to vary greatly from year to year and would not explain the increasing consumption of chlorine. Sunlight causes decomposition of the aqueous chlorine, or hypochlorite, only near the surface of the pool. It was found experimentally that the chlorine near the bottom of the pool remained at a fairly constant concentration. By placing urine in a pool, not only would the chlorine from the surface of the pool be used, but it would also be used from the deeper parts where the sunlight reaction would not be as prominent.

During the summer, the Kjeldahl nitrogen concentration in the pool rose to about one part per million and remained fairly constant. If this nitrogen is due to nitrogen-containing compounds derived from urine, it represents a volume of about 50 gallons of urine in the 680,000-gallon pool. Calculations indicate that this amount of urine could cause a loss of 0.163 ppm chlorine. The nitrogen concentration stabilized because of the high rate of exchange of water in the pool as a result of baskwashing of the sand filters and leakage. The average chlorine concentration in this pool was maintained at approximately 0.50 ppm.

with respect to chlorine, i.e., $\frac{d(Cl_2)}{dt} = k(Cl_2)$.

ALUMINA AS A REDUCING AGENT—A NOVEL SYNTHYSIS OF BENZYL ALCOHOLS

Victor J. Hruby

University of North Dakota, Grand Forks, North Dakota

Department of Chemistry

Chromotography has proven to be very useful for the separation of complex mixtures which are difficult or impossible to separate by the more conventional methods of fractional crystalization, distillation, extraction, etc. One of the most widely used adsorbants for chromatography is activated alumnia. It finds wide use particularly because of its relatively high polarity and thus excellent adsorbing power. Because of its high polarity, however, certain precautions must be taken before using alumina indiscriminately as an ad-

sorbant. For instance, carboxylic acids cannot be chromatographed on alumnina since they cannot be eluted. Dehydration of certain alcohols, Wagner-Meerwein rearrangements, and destruction of unsaturated aldehydes and ketones[1] have been reported to occur on an alumina column. We wish to report another interesting reaction which can occur on alumina, the reduction of benzaldehydes to benzyl alcohols.

Many methods are available for reduction of carbonyl compounds to alcohols. Catalytic hydrogenation, lithium aluminum hydride, the Meerwein-Ponndorf-Verley reduction, and sodium hydride can all be used for carrying out this transformation. The method generally depends on the nature of the carbonyl group present, and the type(s) of other groups present on the molecule to be reduced. Our method is interesting in that it leads to reduction of aldehyde groups α to a benzene ring. Furthermore, the chromatographic technique employed facilitates the ready separation of reactants and products.

General Procedure

The following general procedure illustrates the simplicity of the method employed. Fifteen (15) grams of Alcoa activated alumina (Grade F-20) were packed in a column containing benzene. Then 0.70 grams of the aldehyde or ketone was placed on the column using benzene as solvent. The aldehyde or ketone was then allowed to stand on the column for one hour before elution. Finally the column was eluted with benzene, chloroform, ether, and methanol in the usual chromatographic pattern. The solvents were removed and the products were identified by infrared spectra, melting points, and mixed melting points. In general the unreacted aldehyde or ketone was eluted with benzene, while the alcohol was eluted with chloro-

TABLE I

Reduction of Aldehydes and Ketones on Alumina

Aldehyde or Ketone	Yield of alcohol, pct.	Orig. Mat. recovered, pct.	Alcohol M.P., °C	Literature M.P., °C
p-nitrobenzaldehyde	47.1	40.0	90-91	92[2]
o-nitrobenaldehyde	53.6	45.0	72-73	74[2]
2-4 dichlorobenzaldehyde	58.6	28.6	57-58	56-57[3]
p-chlorobenzaldehyde	48.6	44.3	68-69	72-73
m-nitrobenzaldehyde	50.0	42.9	23-24	27
p-anisaldehyde	21.4	78.6	24.5-25	25
N-N-dimethylamino-benzaldehyde	10.0	87.1	Liquid	(I.R. only)
1-3 dichloro-2-propanone	42.9	37.1	Liquid	(I.R. only)
p-nitroacetophenone	0.0	100.0		
p-nitrobenzophenone	0.0	97.1		
9-fluorenone	0.0	97.1		

form. The results obtained for the various carbonyl compounds used are shown in Table I. In all cases studied, benzaldehydes were reduced. Furthermore the yields of alcohols obtained were roughly in the same order as the carbonyl reactivity series of the aldehydes reduced. Thus the degree of reduction is influenced by other groups present on the benzene ring.

The fact that such reductions can occur is useful simply as a word of warning to those chemists who wish to separate mixtures containing compounds which have the aldehydic functional group attached to an aromatic nucleus. However, we were also interested in an understanding of the other salient aspects of the reaction.

Essentially chromatography on alumina consists of two processes: adsorption of the various compounds on the alumina, and elution of the compounds according to their polarity by choice of a proper solvent scheme. The question is, what part, if any, do these two processes play in the reduction? Are there other processes occuring here, and if there are, what are they?

TABLE II

Reduction of p-nitrobenzaldehyde by Alumina, Effect of Conditions

No.	Condition	Alcohol, pct.	Aldehyde recovered, pct.
1	Aldehyde not allowed to stand on column	14.3	81.4
2	Alcoa Grade F-20 Alumina—1 hour standing	47.1	40.0
3	Aldehyde left on column overnight	65.7	21.4
4	Freeze aldehyde on column with hexane	68.6	31.4
5	Used 30g. of Alcoa Alumina—1 hour standing	81.4	12.9
6	Eluent scheme II (Benzene-ether-methanol)	47.1	45.7
7	Eluent scheme III (Benzene-methanol)	47.1	41.4
9	Eluent scheme IV (Benzene)	0.0	58.6
10	Alumina used third time	22.8	77.1
11	Merck Alumina—#71707	41.4	47.1
12	Merck Acid Washed Alumina—#71695	10.0	87.1
13	Ignited Alcoa Grade F-20 Alumina	62.9	37.1
14	Ignited Merck Acid Washed Alumina—#71695	31.4	57.1

The Role of Adsorption

The first 5 figures on Table II give us some indication of the importance of adsorption in the reduction scheme. By simply allowing the aldehyde longer retention time on the column or by increasing the chance for adsorption by using more alumina or freezing the aldehyde on the column with hexane (in which p-nitrobenzaldehyde is insoluble) we find exactly what might be expected, the greater the adsorption, the higher is the yield of alcohol.

Adsorption is far from the whole story. This is brought out by the fact that ketones (particularly acetophenone) and N-N dimethyl-amino-benzaldehyde are strongly absorbed onthe alumina column, but yield none or very little of the alcohol.

The Role of Solvent

As has been said before, the elution scheme chosen for the general reaction consisted of using benzene, chloroform, ether, and methanol. Other elution schemes were tried and provided some of the most interesting results obtained. Condition 6 of Table II shows the result when we used benzene, ether, and methanol for elution. The yields obtained were almost identical to those obtained in th original scheme. The interesting fact here, is that while the unreduced aldehyde came off with benzene as before, little of the alcohol came off with ether as would be expected if adsorption was the important step in the reduction. The "alcohol" stayed on the column until methanol was introduced. When only benzene was used as eluent, more unreacted aldehyde was recovered, but none of the alcohol could be obtained unless, chloroform or methanol was used later. In both cases, the aldehyde had been adsorbed on the column and could not be eluted by either benzene or the more polar ethyl ether. When chloroform, which is less polar than ethyl ether, or methanol, which is more polar than ether, was added, the alcohol was obtained.

An explanation for these observations is that adsorption does occur, and in fact is very strong (perhaps a reaction occurs), but as soon as a solvent which can furnish active hydrogen is present, the reduction can be completed and the alcohol is eluted. Perhaps the final step in the reduction consists of an exchange on alumina of the adsorbed aldehyde by a solvent molecule somehow denuded of its readily available hydrogen. This explanation is possibly supported by the fact that reuse of the alumina produces a lower yield of the alcohol, presumably because the strong adsorbing positions on the alumina have now been filled with "reacted" solvent. An alternate explanation is that the adsorbing positions on alumina are filled with "unreacted" chloroform which had been used last in the first elution sequence, since, when the alumina was dried in an oven and used a third time, a nearly identical yield of the alcohol was obtained, though it was quite a bit lower then that obtained in the initial chromatography.

Also of interest is the fact that different aluminas lead to different degrees of reduction. The order of reactivity of the aluminas can be explained at the present time by two properties of alumina, that is, the pH of the aluminas, and the amount of volatile material present in the alumina. Table III shows that the orders fit into similar series as those shown in Table II. The amount of volatile material present is an important factor because chromatography on

ignited Alcoa alumina leads to a 25% increase in yield, probably because the alumina is then a better adsorber. Also use of ignited Merck acid washed alumina resulted in over a 20% increase of alcohol obtained. The observation that acidic alumina is less effective then basic alumina for reduction, though it is a better adsorber, is probably an indication of the importance of pH.

In summary, benzaldehydes can be reduced on alumina, but phenylketones cannot. The amount of reduction depends on the type of carbonyl compound, strength of adsorption, proper elution, and type of alumina.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. William Johnson for his encouragement, discussion, and many helpful suggestion during the course of this investigation, and fellow student Leon Royer for his helpful discussions. He is also grateful for the financial aid provided through Dr. Johnson by the National Science Foundation, Grant #G-17345.

TABLE III

Alumina Properties

Alumina	Wt. Loss on Ignition	pH before Ignition	pH after Ignition
Alcoa Grade F-20 Alumina	8.01%	9.73	9.54
Merck Alumina—#71707	9.85	9.45	9.31
Merck Acid Washed Alumina—#71695	15.16	4.24	4.23

BIBLIOGRAPHY

1. E. Lederer and M. Lederer, Chromatography, Elsevier Publishing Company, Amsterdam, Netherlands, 1957.
2. I. M. Heilbron, et. al., Dictionary of Organic Compounds, Oxford Press, New York, New York, 1934.
3. M. Metayer and M. Dat-Xuong, Bull, soc. chim, France, 615-621 (1954); C. A., 49, 6898 (1955).

POSTGLACIAL FRESH WATER-LIMESTONE, MARL, AND PEAT FROM SOUTH-CENTRAL NORTH DAKOTA

Gary G. Thompson

Department of Geology

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

In McIntosh County, North Dakota during June, 1961, an examination of debris recently taken from a dug-out[1] in the SW¼ sec. 24,

T. 132 N., R. 69 W., showed blocks of white marl and chunks of matted but excellently preserved moss. Much of the other sediment in the pile contained abundant small gastropod shells. Water completely filled the dug-out so that the position of the marl and moss in the subsurface could not be observed.

FIELD METHODS

To find the position of the marl and the moss bed, a hole was hand-augered about six feet from the edge of the dug-out. All but 2½ feet of the hole filled with water, making it difficult to retrieve the samples from below the water level. However, by slowly removing the auger from the hole, largely uncontaminated samples were taken.

Twelve samples, taken from different depths, were retained and later analyzed in the laboratory. Approximately 50 grams of each sample were washed through a 200 mesh seive (Tyler Standard) and examined under the binocular microscope.

RESULTS

Description of Sequence

The terms "marl" and "freshwater limestone" are used in this paper for the petrographic types given in Pittijohn's classification of

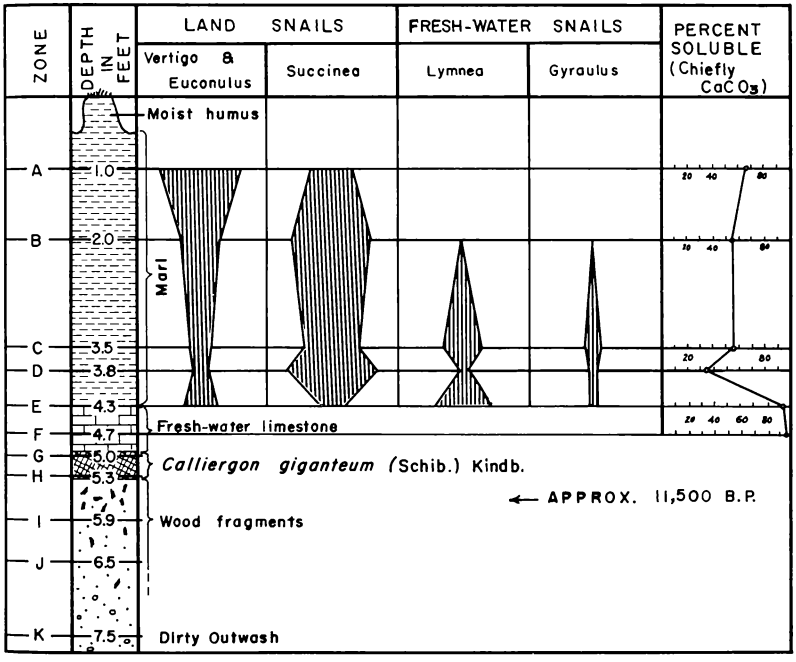


FIGURE 1 — Geologic Section of Deposit

clay-lime mixtures (4). The term "freshwater limestone" describes a mixture with greater than 95 percent calcium carbonate. "Marl" describes a mixture with 35 to 65 percent calcium carbonate.

Upper Gray Marl—The upper few feet, including zones A, B, C, and D are marl ranging in color from medium light to light gray. Gastropods are the only mollusks present but they are abundant. Larger snail shells are fragmented whereas the smaller shells are intact. Those identified[2] in these upper zones are:

Land Snails

Vertigo cf. **V. ventricosta** (Morse)
Euconulus? cf. **E. fulvus**
Succinea cf. **S. grosverni gelida**

Slug

Deroceras sp. ?

Fresh-water Snails

an unidentified lymnaeid
Gyraulus sp.

Sparce **Chara** (stonewort) stems and gyrogonites were found in the upper four zones. Other microscopic calcium carbonate-precipitating algae probably occur in all of these zones since the high calcium carbonate content cannot be attributed to shell material alone. D contains many gray, irregular-shaped, calcareous concretions which could be similar to what Kupsch (2) calls "algal pebbles", from Sturgeon Lake in Saskatchewan. These "algal pebbles" are 1 to 2 cm in diameter and are thought to be concentrations of calcium carbonate precipitated by microscopic blue-green algae.

Fresh-water Limestone—Fresh-water limestone is contained in zones E and F. The limestone of zone E is white when dry and yellowish gray when wet. Zone F is yellowish gray when dry, gray when wet. Insoluble residue analysis of the two zones shows that the color difference coincides with a slight difference in calcium carbonate content.

Mollusks are fairly abundant in zone E which contains the same genera as the higher marl zones. In zone F, only a few lymnaeid snails were found.

Chara remains and other plant fragments and seeds were found fairly abundant in both zones of limestone.

Fresh-water sponges, which are not commonly present in carbonate-rich environments, are evidenced in the limestone by abundant siliceous spicules.

Moss—A sharp contact separates the limestone from the moss in zones G and H. The moss was identified by Dr. Winona Welch of DePauw University as **Calliergon giganteum** (Schimp.) Kindb. It is excellently preserved, appearing to be only discolored to a reddish brown. Fragments and seeds of plants, other than moss, were found throughout the peat.

Mollusks are sparce in these zones. **Sphearium,** a genus of small fresh-water clam, and **Gyraulus** were the only mollusks found in zone G and these were few. Only a few **Gyraulus** occurred in zone H.

Wood—Wood Fragments occur in zones I and J. Zone I consists of wood chips up to 2 cm long, some brown while others are charred black. These fragments grade into a fine black soot in the pebbly J zone at 6.5. Fragments of what are apparently needles and cone fragments indicate that the wood was possibly conifer.

Glacial Drift—Zone K, at the base of the sequence, is a silty ("dirty") gravel containing a few wood fragments. This grades into a normal "clean" outwash gravel below.

DISCUSSION

Topography

The topography near the auger hole is typical of the Missouri Coteau. It is characterized by low hills, closed depressions, and completely unintegrated drainage. The hole is in a gentle slope approximately 300 yards from a present-day slough.

Stratigraphy

An undetermined thickness of upper Wisconsin outwash underlies the auger hole sediments. Lineated "push moraine", resulting from the bulldozing action of a slight readvance of Burnstad (late Wisconsin) ice, are present about a mile east of the hole location. To the west, lake sediment of Glacial Lake Lehr grades into the outwash approximately 2 miles from the hole.

Two carbon-14 determinations date the drift of this area. Moir (3) reported a date of 11,480 ± 300 years before present (sample W-542) on a spruce stump that was rooted in wind-blown sand derived from outwash in front of the Burnstad end moraine in Kidder County, North Dakota (approximately 40 miles to the northwest). A second date of 11,650 ± 310 years B. P. (sample W-947) has been recently reported on clam shells from a lens of marl in the "push moraine" mentioned above.

The horizon on which the spruce stump occurred in Kidder County appears to be much the same as the zones containing wood fragments in the auger hole. Zone K is apparently outwash that has been altered by slope wash or wind action. Sample K contains about 30 percent silt and clay. This amount of silt and clay is much less than the fine fractions of Burnstad till and much greater than the fine fractions of uncontaminated outwash in this locality. It is most probable that wind or slope wash brought in fine material to contaminate the outwash. Winnowing fines from till would require higher energy conditions than could exist here. It would seem, then, that zone K and the spruce horizon in Kidder County are stratigraphically similar and were deposited at approximately the same time. Together with the evidence given by the date from the "push moraine", this would indicate that the auger hole sequence bgan about 11,500 years ago.

Sequence of Events

The wood fragments of the lower zones appear to have been washed a short distance by slope wash and accumulated in or near the edge of very shallow water. Upward from these zones there are evident changes in the depth of water during deposition. Because of the permeability of the underlying outwash, the fluctuation in water level would be due to a rising and falling water table. A possible mechanism for such a water table is presented below.

Immediately above the wood fragments, the moss bed indicates shallow water of some permanence. **Galliergon giganteum** (Schimp.) Kindb. is aquatic to subaquatic, often being nearly submerged in deep loose masses in cool swamps across the northern United States and Canada (Dr. Winona H. Welch, written communication). This would indicate slightly cooler temperatures and moister conditions since there are no true cool swamps in this area today. Moir (3) made similar inferences from the fossil spruce in Kidder County. He states:

> "The presence of spruce in this area, not necessarily as continuous forest cover but more probably in scattered groupings in the more favorable habitats, suggests a climate cooler and moister than that experienced today."

The sharp contact of the peat with the limestone above indicates a sudden change in depositional conditions. In the normal filling of a lake or marsh, as the water depth decreases; limestone formation ceases and moss begins to flourish. In the auger hole, the sequence is reversed—limestone overlies the moss. Apparently the water was getting deeper.

The processes involved in the deposition of fresh-water limestone here bear consideration. Of the possible processes contributing to the formation of fresh-water limestone and marl, biochemical precipitation by **Chara** and microscopic algae is the most important (1). Kupsch (2) has shown that **Chara,** blue-green algae, and diatoms make up about 90 percent of a Saskatchewan fresh-water limestone. This is comparable to the results shown in Fig. 1 for zones E and F.

The optimum conditions for the formation of fresh-water limestone are outlined by Kupsch (2):

1. Coarse texture of till and other glacial deposits which are the ultimate source of the calcium carbonate. A coarse texture will facilitate groundwater circulation responsible for the transportation of the marl [fresh-water limestone].
2. A high percentage of soluble carbonates in the till.
3. A rugged topography surrounding the lake of marl deposition. This factor increases groundwater circulation. It is present in the Sturgeon River Valley as the valley is about 200 feet below the surrounding plane.

In the area of the auger hole, conditions (1) and (2) are duplicated. The outwash offers perfect medium for groundwater circula-

tion. In the pebble size of the surrounding drift, limestone and dolomite make up approximately 60 percent of the total, thus providing a source for the carbonate.

The third condition, requiring high relief, was probably satisfied in a different way. Remnant blocks of stagnant ice, which had become detached from the main glacial margin as it retreated and were covered by an insultating layer of ablation drift. This ice probably produced the necessary head by contributing meltwater in the subsurface by seepage.

The sudden truncation of the limestone bed indicates another change in environmental conditions. The amount of calcium carbonate being precipitated suddenly dropped. As the stagnant ice blocks melted, the amount of groundwater supplied by them decreased and less solution of calcium carbonate from the drift resulted. With less carbonate available to them and with a less stable water table, algae did not fluorish as before and precipitation of calcium carbonate was less intense.

Another suggestion as to the change in calcium carbonate content of the sediments is that of a process involving seasonal arid conditions. The precipitation by evaporation of upward moving groundwater may have caused much of the marl deposition. In any case, a larger percentage of fine detrital material resulted.

From the top of the fresh-water limestone to the upper, zone A, the snails were identified and the percentages of each species were plotted against depth (See Fig. 1).

Demonstrated in Figure 1 is the change in moisture conditions as refelected in the difference in dominant species of snails at different levels. The inferred environmental conditions are based on the ecological distributions of these species today. **Vertigo** and **Euconulus** are land snails found usually in well-shaded places among damp leaves and under dead wood. **Succinea** ranges from swampy areas to the humus of relatively dry regions (5). The lymnaeids and **Gyraulus** are found in temperary to permanent ponds (6). The other species are numerical insignificant.

Zone E, which contains part of the limestone and a dominance of the fresh-water snails, indicates relatively deep water.

Lower calcium carbonate content and a high percentage of **Succinea** in zones C and D show a time of shallow and temporary water in which a few fresh-water species survived.

Zones A and B represent dryer conditions with the disappearance of the fresh-water snails. The increase in calcium carbonate content appears anamolous when considered to be due to algal precipitation alone. Perhaps a part of the carbonate is due to some seasonal arid conditions such as mentioned above.

FUTURE STUDIES

Further studies in this area, especially where many undrained sloughs occur, should uncover excelent materials for detailed paleo-

climate reconstruction. Pollen is undobtedly abundant in the sediments. A pollen profile might give a much clearer reconstruction of the events indicated in this paper.

ACKNOWLEDGEMENTS

The samples were collected while the author was employed as a student assistant for the North Dakota State Geological Survey, Dr. Wilson M. Laird, Director. Mr. Lee Clayton, offered many valuable suggestions. Mr. Samuel J. Tuthill, of the University of North Dakota, identified the molusks mentioned in this paper. Dr. Vera Facey, of the University of North Dakota, gave many helpful suggestions for examining the flora of the sequence. Dr. Winona H. Welch of DePauw University, Greencastle, Indiana, contributed valuable information and service in identifying the moss.

LITERATURE CITED

1. Davis, C. A., 1900, A contribution to the natural history of marl: Jour. Geol., v. 8, p. 485-497.
2. Kupsch, W. O., 1956, Geology of the Sturgeon Lake Marl Deposit, Report of Invesigations No. 8, Saskatchewan Dept. of Mineral Resources, p. 1-25.
3. Moir, D. R., 1958, Occurrence and radiocarbon date of coniferous wood in Kidder County, North Dakota, Guidebook Ninth Annual Field Conference Mid-western Friends of the Pleistocene: North Dakota Geol. Survey, Misc. Ser. no. 10, p. 108-114.
4. Pettijohn, F. J., 1957, Sedimentary Rocks. New York, New York, Harper and Brothers. 718 p.
5. Pilsbry, H. A., 1939, 1940, 1946, 1948, Land Mollusca of North America (north of Mexico), Acad. Nat. Sci. Philadelphia.
6. Taylor, D. W., 1960, Late Cenozoic Molluscan Faunas from the High Plains: U. S. Geol. Survey Prof. Paper 337, 300 p.

[1]A dug-out is an excavation on flat ground which fills with water by seepage and is used for watering livestock.
[2]Identification was done by Mr. S. J. Tuthill.

IDENTIFICATION OF A HEAT-LABILE, CHROMOGENIC MATERIAL IN FASTED RAT PLASMA WHICH AFFECTS CERTAIN CITRATE DETERMINATIONS

Bruce D. Stainbrook and Roger B. Meintzer

Agricultural Biochemistry Research Laboratory

Department of Biochemistry

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

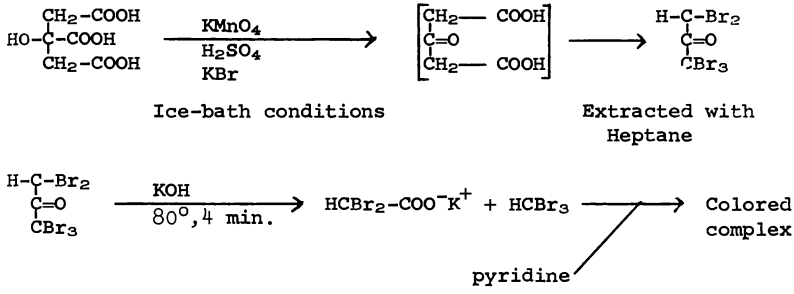
Methods for the determination of citric acid in biological materials all involve the oxidation and bromination of citrate to yield

pentabromoacetone. Studies on the oxidation and bromination of citric acid were reported as early as 1847 by Cahours (1). Since then many qualitative and quantitative procedures have been developed which attempted to measure micro-quantities of critric acid using this general procedure.

Natelson **et al.** (2) described a colorimetric method for the determination of citric acid in which pentabromoacetone is reacted with thiourea to form a yellow-colored complex. The method is reportedly accurate to within ± 5% for amounts ranging from 1 to 20 μg. of citric acid.

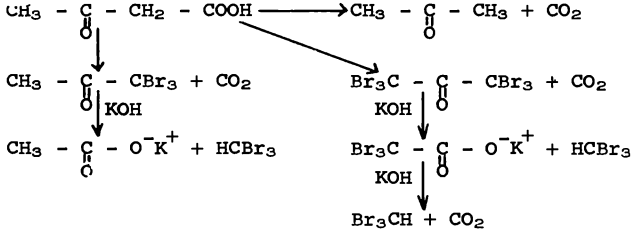
Ettinger **et al.** (3) described a simplification of Natelson's procedure for the oxidation and bromination of citric acid to pentabromoacetone. In this method the reaction of trihalogenated hydrocarbons with pyridine, as described by Fujiwara (4), was adapted to the measurement of the pentabromoacetone. This photometric procedure measures citric acid in the range of 2 to 40 μg. and is accurate to within ± 5%. (Figure 1).

Figure 1: Chemical reactions in citrate determination by Ettinger et al.



Conversion of citrate to bromoform, heated to give color, gives 85-87 percent of theoritical color possible under standard conditions.

Figure 2: Chemical reactions in acetoacetic acid determination.



Ettinger further reported that metabolic intermediates of the citric acid cycle in the blood produce no detectable pentabromoacetone at concentrations equivalent to blood levels of 500 μg. per ml. At concentrations equivalent to blood levels greater than 2000 μg. per ml., a small but measurable amount of color was reportedly produced by acetoacetic acid and β-hydroxybutyric acid but none by glucose or fructose.

In studying citrate metabolism in our laboratory blood plasma from fasted rats was found to give very high citrate values which could not be duplicated on re-analysis. Upon examination of this phenomenon it was realized that a labile, chromogenic material was present. This material was destroyed by heating under conditions in which citrate was stable. It was our purpose to identify this labile material.

RESULTS AND DISCUSSION

It is well known that the β-keto acid, acetoacetic acid, accumulates in the blood stream of fasted animals. It is heat-labile and should behave chemically like β-ketoglutaric acid which others have suggested as an intermediate in the formation of pentabromoacetone from citrate and which gives color equivalent to an equimolar quantity of citrate in citrate determinations. The decarboxylation product of acetoacetic acid, acetone, was shown by Ettinger **et al.** (3), and was found also by us, to produce no color in the citrate determination. Thus acetoacetic acid meets the requirements for the chromogenic, heat-labile material we sought to identify.

Originally we tested the most readily available form of acetoacetic acid, namely its ethyl ester, and found that it gave a measureable amount of color which could be increased by prolonging the period of oxidation and bromination or by performing the reaction at room temperature rather than at ice-bath temperatures. It was apparent that, under acid conditions, ester hydrolysis was occurring and the amount of color produced was increased by permitting greater ester hydrolysis.

We next hydrolyzed the ester prior to incorporation into the citrate analysis and found that a large amount of color was produced. Results from hydrolysis time studies are shown in Table I. It is seen that a period of two hours of hydrolysis was required for maximum color formation while a longer period resulted in decreased color formation because of decarboxylation of the unstable acid. It is also seen that more than an equivalent amount of bromoform was produced under optimum conditions.

To get quantitative data acetoacetic acid was prepared by the method of Krebs (5) and kept frozen at −18°C. Krebs had shown that this frozen, aqueous preparation decomposes only to the extent of 5% in a month's time. This solution was assayed manometrically using the method of Edson (6) in which aniline citrate is used to

TABLE 1

Results obtained from the hydrolysis of ethyl acetoacetate with an equivalent amount of KOH.

Time of Hydrolysis	"Citric Acid Assay" of acetoacetic acid equivalent to 20 μg. of citric acid.
Hours	μg. of citric acid
0.5	12.0
1.0	20.2
1.5	22.8
2.0	26.0
4.0	22.0

Preparation of Solution: 6.21 ml of ethyl acetoacetic ester per 500 ml. of H_2O and KOH (2.7g). Aliquots were taken from the solution at indicated time periods and analyzed by the citric acid method of Ettinger **et al.**

TABLE II

Effect of fasting on response to nephrectomy of plasma citrate and heat-labile, chromogenic material.

Rat Group	Acetoacetic Acid, mg/100 ml.	Acetoacetic Acid as determined by citric acid assay, mg/100 ml.
—D		
1	6.2	5.7
2	4.6	4.7
3	5.6	6.2
4	8.8	8.5
5	3.6	3.8
6	3.8	4.0
	5.4 ± 1.9	5.6 ± 2.1
+D		
1	5.4	5.8
2	8.4	8.1
3	8.6	8.2
4	7.2	6.7
5	8.0	8.2
6	6.4	6.1
	7.3 ± 1.2	7.2 ± 1.0

Rats used received diet 3AMR (a normal calcium and phosphorus containing diet).

Animals were nephrectomized four hours since this produces a greater accumulation of labile material.

quantitatively decarboxylate the acetoacetic acid. The CO_2 evolved is measured and from it the concentration of acetoacetic acid can be calculated. Later, solutions of lithium acetoacetate were used as standards in obtaining quantitative data. This preparation was recently described by Hall (7).

Citrate analysis of acetoacetic acid standardized solutions did not always give the same amount of color presumably because of competing reactions. The time involved between addition of lithium acetoacetate solution and the $KMnO_4$ and KBr addition was more important than the temperature of reaction in determining the color produced. Maximum color was obtained when the acetoacetic acid solution was added directly into the chilled oxidizing and brominating mixture. Under optimum conditions for color formation, 137% of the theoretical amount of color was produced when calculated on the basis on one bromoform produced per mole of acid. The course of reaction of acetoacetic acid must, of necessity, involve production of more than one mole of bromoform from a mole of acetoacetic acid to account for the color formed since at least three halogens per carbon were found essential for color formation. (Figure 2).

Pentabromoacetone is not formed from acetoacetic acid since the citrate method of Natelson **et al.** (2) which is specific for pentabromoacetone gave no color from acetoacetic acid. The labile material in fasted rat blood plasma also did not produce pentabromoacetone as shown by determination using the Natelson method with heated or non-heated plasma samples.

Finally, quantitative tests were made for acetoacetic acid in fasted rat plasma using the colorimetric procedure of Walker (8). These results were compared with the value obtained from color formation with the heat-labile, chromogenic material in fasted rat plasma using the citrate determination of Ettinger (3) under carefully controlled conditions. The latter value was obtained by subtracting the optical density obtained with plasma samples, which were heated at 80° for 10 minutes to detroy the heat-labile material, from the optical density obtained with a similar amount of unheated plasma. The amount of color produced by a known amount of acetoacetic acid was used to convert optical densities to μg. of acetoacetic acid. The results are shown in Table 2.

Although the heat-labile, chromogenic material hasn't been isolated and characterized—a task which would be extremely difficult—all of the properties of the material have been shown to be similar to those of acetoacetic acid.

It can therefore be said, that it is essential that biological samples analyzed for citrate by the Ettinger method be heated to remove this labile material. Additional study of the oxidation and bromination of acetoacetic acid under these conditions is being made by others.

SUMMARY

Through comparative studies of chemical behavior a heat-labile, chromogenic material which produces color in the citrate assay of Ettinger **et al.** and which appears in fasted rat plasma has been identified tentatively as acetoacetic acid. In using this assay it is essential that biological samples be heated to destroy this material before analysis.

LITERATURE CITED

1. Cahours, A., Ann. Chim., **19,** 484 (1847).
2. Natelson, S., Pincus, J. B., and Lugooy, J. K., J. Biol. Chem., **175,** 745 (1948).
3. Ettinger, R. H., Goldbaum, L. R., and Smith, L. H. Jr., J. Biol. Chem., **199,** 531 (1952).
4. Fujiwara, K., Silzungaber. Naturforsch.—Ges. Rostock, **6,** 33 (1916); C. A. **11,** 3201.
5. Krebs, H. A. and Eggleston, L. V., Biochem. J. **39,** 408 (1945).
6. Edson, N. L., Biochem. J. **29,** 2082 (1935).
7. Hall, L. M., Anal. Biochem., **3,** 75 (1962).
8. Walker P. G., Biochem. J., **58,** 699 (1954).

THEORETICAL PREDICTION OF THE PROPERTIES OF COMPOUNDS PART I. STABILITY RULES FOR PLANAR CONJUGATED RING SYSTEMS

H. S. Lee

Department of Chemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In 1931 Hückel first applied the so-called molecular orbital method to calculate the delocalization energy of benzene. Information obtained from molecular orbital calculation has been used to explain various properties of molecules, such as bond lengths, resonance energy, π-π^* electronic transition, and chemical reactions. At present the instability of compounds such as cyclobutadiene and benzocyclobutadiene . . . has not been explained. Cyclobutadiene has zero delocalization energy with a triplet ground state according to the MO treatment. This compound has not been prepared. Since the known

cyclopropene should have greater angular strain, the instability of this monocyclic polyene has been ascribed to the triplet ground state. Likewise molecular orbital calculation on benzocyclobutadiene indicates that this bicyclic compound has DE 2.381β with $^1\Sigma$ ground state. Apparently this polyene should be capable of existence, but it has not been isolated.

Since the instability of some compounds has not been explained, the predictions which have appeared in the literature about the properties of these compounds are not entirely reliable. For example, MO treatment on bicyclohexatriene indicates that this substance has DE 1.656β with singlet ground state and does not have high F-values. According to the DE criterion or the F-value criterion this compound should have synthetic stability. Isolation of this polyene has not been reported. Similarly a number of ingenious attempts at synthesis of the derivatives of benzocyclobutadiene have not been able to yield positive results. These and other unsuccessful attempts at synthesis of unknown compounds strongly indicate the demand for a correct explanation of the stability of ring compounds and for a reliable prediction of the properties and stability of new ring compounds. Seven stability rules are proposed by which the stability of ring compounds can be explained and predicted.

The stability rules for planar conjugated ring systems may be stated as follows:

RULE I

The more delocalized energy a system has, the more stable the system will be, provided this system is not greatly destabilized by one or more of the instability factors such as:

(1) large angular strain
(2) zero DE or small DE
(3) high free valence index
(4) non-singlet ground state
(5) steric hindrance arising from interior hydrogens
(6) small energy difference between the highest occupied molecular orbital and the lowest empty molecular orbital
(7) all bonding molecular orbitals have not been completely occupied or have electrons in the antibonding molecular orbital
(8) high degree of double bond fixation (for bonds involving a highly strained ring)

RULE II

For "synthesis" of system B from system A by increasing the extent of conjugation, either by increasing the number of conjugated carbon atoms two at a time or by adding additional bonds one at a time to the same conjugated system,

$$A \longrightarrow B$$

the "synthesis energy" $(\Delta E)_{BA}$ is defined as

$$(\Delta E)_{BA} = (DE)_B - (DE)_A$$

(Note: the word "synthesis" used here does not imply any actual experimental method of preparation).

A "synthetic" route is said to be a "stable synthetic" route if the value of "synthesis energy" in every step of this route is positive, otherwise the "synthetic" route is said to be an "unstable synthetic" route. A system "synthesized" via an "unstable synthetic" route is called an "unsynthesizable" system.

In a "synthesis" diagram, some systems might appear to be "synthesizable", others not. Some systems might appear to be both "synthesizable" and "unsynthesizable".

RULE III

If a system is not "synthesizable" from any one of the possible "synthesizable" systems, then this system can be predicted to be incapable of existence because of its instability.

RULE IV

If a system is "synthesizable" only from "unsynthesizable" systems, then this system can be predicted to be incapable of existence.

RULE V

For systems "formed" by fusion of two or more "unsynthesizable" systems, in general it can be predicted they will not be capable of existence because of their unstaibilities.

RULE VI

If a system is "synthesizable" from all possible "synthesizable" systems, then the capability of existence of this system depends on the degree of influence of the instability factors stated in rule I.

RULE VII

All atoms in a molecule, jointed directly or indirectly, mutually interact with one another. Each atom in a molecule has a definite chemical interaction with the others, and hence each part of the molecule affects the rest of the molecule. The influence of interaction among the atoms in a molecule reaches a certain equilibrium. In a planar conjugated system there exists a delocalized strong π bond, through which the stable part of the molecule affects and hence stabilizes the unstable part of the molecule. A system, therefore, "formed" by fusion of an unstable system and one or more stable systems can be predicted to be capable of existence provided that it has large enough stabilization energy and that it is not greatly destabilized by the instability factors stated in rule I. The stabilization energy ΔE_S is defined as follows:

$$\Delta E_S = \underset{\text{system}}{(DE)} - \underset{\text{stable parts}}{(DE)}$$

The minimum required stabilization energy for each carbon atom of an unstable four-menbered ring, of which all four carbon atoms

are sp^2, is assigned to be 0.114β. Componnds containing one or more unstable rings, therefore, are capable of existence if they have the required minimum stabilization energy and are not greatly destabilized by the instability factors stated in rule I.

By using group theory, via molecular orbital method, the DE, bond order, F-values, electron densities and charge dencities of more than one hundred and fifty unknown ring compounds have been calculated. Most of the calculations have been made with neglect of the exchange integrals between non-adjacent atoms and of non-orthogonality of atomic orbitals on different nuclei. From the results of the calculations, with the explanation and prediction of the stability rules, the properties of these unknown substances can be theoretically foretold. Since the amount of work is too large to be reported in one paper, the present article is condensed to the simple statement and brief discussion of the stability rules.

BIBLIOGRAPHY

1. E. Huckel, Z. Physik, **70,** 204 (1931).
2. C. A. Coulson, Proc. Roy. Soc. **A169,** 413 (1939).
3. G. W. Wheland, J. Chem. Phys., **2,** 474 (1934).
4. J. R. Platt, H. B. Klevens, and W. C. Price, J. Chem. Phys., **17,** 466 (1949).
5. K. L. Nelson, J. Org. Chem., **21,** 145 (1956); R. D. Brown, J. Chem. Soc., **1950,** 3249; **1951,** 1950.
6. C. A. Coulson and W. E. Moffitt, Phil. Mag., (7) **40,** 1 (1949).
7. J. D. Roberts, A. Streitwiesser, Jr., and Clare M. Regan, J. Am. Chem. Soc., **74,** 4580 (1952).
8. Theoretical prediction of the properties and hence the stability of these 150 compounds will be reported in separate papers.
9. E. Clar, Aromatische Kohlenwasserstoffe, Springer-Verlag, Berlin (1952), p. 52.
10. R. F. Curtiss, J. Chem. Soc., 3650 (1959).

A BRIEF HISTORY OF CHEMICAL WEED CONTROL IN NORTH DAKOTA

(Invited Paper)

E. A. Helgeson

Department of Botany

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

The first chemical weed control in the United States was started at this station in 1897 under the direction of H. L. Bolley and continued for some twelve or more years. In 1908 he published an ex-

tensive bulletin covering these researches (1). Bolley's choice of chemicals was limited to ferric sulphate, several copper salts, sodium chloride and some arsenicals. He demonstrated that broad-leaved weeds such as mustard and marsh elder could be killed by contact sprays whereas the narrow leaved cereal weeds and the waxy leaves of flax effectively shed these aqueous solutions.

Two factors prevented the wide spread use of this new means of weed control in our large acres of cereal fields. First, large volumes of water (around 50 gallons) were required per acre and second, the sprays dried too quickly to cause the necessary fatal burning of leaves and stems.

After a lapse of many years Barnett and Hanson (2) published an extensive study of leafy spurge control with the then popular sodium chlorate spray. They concluded that this chemical was an effective control measure but too expensive for large infestations.

Since 1936 the present writer has been in charge of chemical control experimentation of weeds on a part time basis. The remainder of this paper will be divided into (A) the practical field aspects of and recommendations for control with the various new chemicals which have been made available throughout the ensuing years and (B) with a separate section dealing with basic physiological studies on weeds or herbicides.

Beginning about 1949 and continuing for a period of some five years a physiologist or agronomist from the U.S.D.A., A.R.S. Division was also stationed here to assist in our weed studies. Further, it should be made clear that while most of the chemicals listed were tested at our station, replication by fellow workers in the NCWCC beginning in 1945 was used to lend confidence to our recommendations on the use of chemicals for selective weed control in crops.

A. Field Studies on Weed Control.

In 1940 the writer published a bulletin on the control of perennial peppergrass and Russian knapweed (3). This study showed that knapweed could be readily controlled whereas the peppergrass was quite resistant to sodium chlorate.

Our first study on the control of annual weeds in flax or in grain fields with Sinox, a new dinitro weed killer, was performed in 1940. This chemical readily controlled broad leafed weeds such as mustard without injury to the accompanying crop. Here again, however, large quantities of water were needed (4).

The use of chlorate sprays has always been hazardous and this prompted an investigation into the use of the chemical applied as a dry crystal at various times of the year. Fall applications of dry chlorates proved to be effective in control of bindweed and other perennial weeds thereby eliminating the hazard from spray (5).

In 1945 a new chemical known as 2,4-D was introduced for trial on broad leafed weeds. There were good indications that it might

prove effective on some of our harder to kill perennials such as the Canada thistle (6).

In 1948 our first extensive circular recommending the widespread use of 2,4-D on annual and perennial broad leafed plants was published (7).

In 1948 a new grass killer, TCA, appeared promising while 2, 4, 5-T and ammonium sulfamate were being tried for stump, brush and sprout control.

Our first recommendation for grass control in sugar beets and small seeded legumes with TCA was made in 1952. At that time another hormone chemical, MCP, appeared promising for broad leafed weed control in flax and certain legumes (8).

In 1953 a series of new substituted ureas CMU, etc. were recommended as permanent soil sterilants.

Considerably expanded use of such chemicals as 2,4-D, MCP, 2, 4, 5-T and some of the new boron and boron chlorate mixtures were recommended for widespread use in crop and non-crop land. (9)

In 1957 a new grass killer based on a chlorinated propionic acid was suggested for use in seedling stands of legumes and in sugar beets for control of grassy weeds. ATA for Canada thistle control and ammate for spurge control was also recommended (10). This circular also carried the statement concerning food and drug administration precautions and mentioned that the dinitros, 4-(2, 4-DB), TCA and Dalapon could not be used on hay and pasture crops.

After several years of testing, two new wild oat killers, which can be used selectively in flax and in some cereal crops as well as in sugar beets and potatoes, were recommended for use. These are carbamates—the one known as Avadex and the other as Barban. In addition to these a number of soil sterilants which are mixtures of benzoic compounds alone or with certain boron compounds and several triazines were recommended in 1962 for small areas of perennial weeds and as somewhat permanent soil sterilants (11).

It should be mentioned that beginning about 1948 new improved spray rigs capable of applying as little as 5 gallons of spray per acre made possible the marked success obtained with the new hormone chemicals. It also should be brought to mind that where Bolley had at the most a choice of 5 chemical compounds the present weed worker has at his command some scores of new compounds or mixtures thereof. Further many of these new compounds are systemic and kill because they penetrate and completely diffuse throughout the entire plants. Where Bolley used 100 pounds of iron sulphate per acre we can now achieve the same results with as little as 2 ounces of 2, 4-D.

B. Basic Physiological Studies of Weeds or Herbicides

For some time it had been noted that the control of perennial weeds with chlorates was less effective in highly fertile soils. In 1939

Crafts reported on this particular phenomenon. Our studies along similar lines indicated that heavy application of nitrates would antagonize the effect of chlorates (12).

With the introduction of 2, 4-D for weed control in spring wheats the question of the chemical effect on the milling and baking qualities of sprayed wheats was raised. Extensive studies on the effects of several 2, 4-D compounds applied at various rates and at several growth stages demonstrated that where heavy applications of ester formulations might be detrimental the ordinarily recommended rates for weed control were generally unimportant. In a number of instances the protein content was increased in some varieties. As far as we are aware this was the first detailed study on these factors (13).

At about the same time, it was suggested that perhaps the selective action of 2, 4-D might be explained in part by metabolic effects on the plant. Work with castor bean lipase showed that 2, 4-D inhibited the activity of this enzyme at very low concentrations. The butyl ester of 2, 4-D was shown to be inactive as an inhibitor for the lipase and that it must first be hydrolyzed to the 2, 4-D acid for activity (14).

In our extensive trials on the effects of various herbicides on the germination and early seedling development, it soon became evident that extracts from various weed tissues were also toxic. Aqueous extracts of field bindweed and Canada thistle proved to be highly toxic to flax and wheat seedlings (15).

Extensive studies were initiated in 1953 and continued to the present time on the dormancy of the wild oat seed. In 1957 we demonstrated that gibberelic acid and its potassium salt were effective in breaking the dormancy of wild oats. Whereas freshly harvested seeds required about 500 ppm to break dormancy, 50 ppm were effective after 6 months' storage. This seems to indicate that unknown chemical changes occur as time elapses (16).

It has been recently demonstrated that aqueous extracts of wild oat seeds and seedlings will also break the dormancy of wild oat seeds to a degree comparable to gibberellic acid (17).

In field spraying of wild oats with dalapon during the later stages of seed development, the chemical seems to accumulate and cause sterile seeds or malformed seedlings (18). A recently completed study indicates that endothal has a similar effect (19).

LITERATURE CITED

1. Bolley, H. L., 1908. Weed control by means of chemical sprays. N. Dak. Agr. Expt. Sta. Bull. 80:541-574.
2. Barnett, H. L. and H. C. Hanson, 1934. Control of leafy spurge and review of literature on chemical weed control. N. Dak. Agr. Expt. Sta. Bull. 277.
3. Helegson, E. A., 1940. Russian knapweed and perennial peppergrass. N. Dak. Agr. Expt. Sta. Bull. 292.

4. Helgeson, E. A. and D. Gerbracht, 1940. Chemical control of annual weeds in flax and grain fields. N. Dak. Agr. Expt. Sta. Bimo. Bull. 3(2):7-10.
5. Helgeson, E. A., 1941. Control of field bindweed by dry chlorates. N. Dak. Agri. Expt. Sta. Bimo. Bull. 4(1):7-8.
6. ———, 1945. Suggestions for control of Canada thistle. NDSU Ext. Serv. Circ. A-89.
7. Jensen, L. A. and E. A. Helgeson, 1948. 2, 4-D for weed control. NDSU Ext. Serv. Circ. A-108 (Rev.).
8. Helgeson, E. A., D. E. Kratochvil, and R. B. Widdifield, 1952. Weed control with T.C.A. and M.C.P. NDSU Ext. Serv. Circ. A-175.
9. Helgeson, E. A. and R. B. Widdifield, 1953. Chemical weed control in growing crops. NDSU Ext. Serv. Circ. A-190, 1953.
10. Jensen, L. A. and E. A. Helgeson, 1959. Chemical weed control in field crops. NDSU Ext. Serv. Circ. A-253 (Rev.); 1957; Circ. A-253.
11. Jensen, L. A. and E. A. Helgeson, 1962. Chemical weed control in field crops and for perennial weeds. NDSU Ext. Serv. Circ. A-253.
12. Helgeson, E. A., 1940. Nitrates in relation to the toxicity of sodium chlorate N. Dak. Agr. Expt. Sta. Bimo. Bull. 3(1):9-10.
13. Helgeson, E. A., K. L. Blanchard, and L. D. Sibbitt, 1948. The effect of three 2, 4-D compounds on yield and quality of North Dakota wheats. N. Dak. Agr. Expt. Sta. Bimo. Bull. 10(5):166-171.
14. Hagen, C. E., C. O. Clagett and E. A. Helgeson, 1949. 2, 4-Dichlorophenoxyacetic acid inhibition of castor bean lipase. Science 110-(2848):116-117.
15. Helgeson, E. A. and Richard Konzak, 1950. Phytotoxic effects of aqueous extracts of field bindweed and of Canada thistle—a preliminary report. N. Dak. Agr. Expt. Sta. Bimo. Bull. 12(3):71-76.
16. Helgeson, E. A. and John G. Green, 1957. New weapon against wild oats. N. Dak. Agr. Expt. Sta. Bimo. Bull. 19(4):121-122.
17. ———, 1958. Gibberellic acid vs. wild oats. N. Dak. Agr. Expt. Sta. Bimo. Bull. 20(7):7-8.
18. Anderson, Robert N. and E. A. Helgeson, 1958. Control of wild oats by prevention of normal seed development with sodium 2, Dichloropropionate. Weeds 6(3), 1958, pp. 263-270.
19. Helgeson, E. A. and Harley Swanson. Unpublished data. 1962.

THE FLIGHT ACTIVITIES OF FORMICINE ANTS[1]

Paul B. Kannowski[2]

Department of Biology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT[2]

The reproductive female and male ants, which are produced in mature colonies only once or twice a year, usually leave the parent

colony by flying into the air on "nuptial flights." It is through the flights that the two functions, reproduction and dispersal, take place.

The typical sequence of activities relating to flights is as follows: (1) preparation of the nest for emergence; (2) emergence of alates; (3) positioning of alates for flight; (4) flights; (5) pairing; (6) copulation; (7) separation; (8) male flight and possible additional matings; (9) female grooming and possible additional matings; (10) dealation of the female; (11) location of colony site by the females; (12) nest construction; (13) egg laying.

Observations of alate behavior during the flight seasons of several species of **Acanthomyops, Brachymyrmex, Formica, Lasius, Polyergus,** and **Prenolepis** are discussed. The time of the year at which alates of a given species are matured in nests is approximately the same each year; variation in timing of this annual rhythm in diverse parts of a species range appears to be due primarily to climatological factors. The time of the day at which the alates emerge from the nests prior to flights seems, in most cases, to be rhythmical. Environmental factors that modify the actual time of flight are usually temperature and light intensity. The flight period of most formicine ants is in the afternoon or early evening. In most formicine taxa, the flights do not occur until most, if not all, of the alates have matured in the nest. Hence, the flights are frequently conspicuous.

The first indication of the approaching flight season comes with the enlargement of the nest entrances. The emergence of alates from a nest is often controlled by workers, especially in colonies with large alate populations. The alates usually fly from the top of vegetation on or within a few feet of the nest. Prior to flight, the alate usually flutters its wings briefly, after which it sometimes moves to a new position on the vegetation and flutters again. At take off, the alate may flutter for several seconds, then release its hold on the vegetation. At other times, the release of the vegetation appears to occur with the first strokes of the wings.

Aerial swarming is uncommon in Formicinae but has been observed of certain species of **Formica** on mountain tops. Ground swarming has been observed of certain species of **Formica** and **Prenolepis.** The males of several species of **Formica** fly just above the vegetation apparently seeking females on the vegetation at or near the female nest. This male behavior is termed "patrolling." Mating is rarely observed unless it occurs on the ground or vegetation. The mating behavior of **Formica subintegra** is described.

---

1. Supported by research grants from the University of North Dakota Faculty Research Fund, The Society of the Sigma Xi, and the National Science Foundation (#G-12041).

2. The complete paper has been published in **Symposia Genetica.**

PROCESSING OF LIGNITE TO COMMERCIAL PRODUCTS

Carlos M. Henderson[1] and Stanley V. Margolin[2]

Rubber extenders, timber preservatives, flotation oils, disinfectants, weed killers, antiseptics, plastics, fuels, barbecue briquets—these are the products of today and the future which could foreseeably be produced on the rolling plains of North Dakota.

Today, Husky Briquetting (Division of Husky Oil Company), Dickinson, North Dakota, is producing on a year-round basis barbecue briquets to fire the vast assortment of broilers, rotisseries, hibache ovens, grates and grills in the United States and Canada. This year, these bags have appeared or will appear in market places—grocery stores, supermarkets, hardware stores, gas stations and fruit stands. These bags contain the Husky briquet made from lignite, successful culmination of a two year research program. Tomorrow will see some of these products of today's research.

This paper will present the story behind the research program for development of products from lignite, a description of the Dickinson facility and a prognostication of the future of processing of lignite in North Dakota.

History of Dickinson Operation

The story really begins over five years ago and is concerned with crude oil, or more accurately the lack of known crude oil reserves.

The Korean Conflict indicated a need to supplement crude oil with another source of liquid hydrocarbon. The Husky Oil Company in one of its research programs started a study of coal carbonization to supply these fuel needs.

As the study for fuel recovery progressed, it was found that a high grade metallurgical coke for non-ferrous metals could be produced from lignite char. Research was started in this very important field. During this study, the Husky research team found hidden among many compounds of the lignite char materials which they felt would provide an improved barbecue fuel.

To take advantage of the burgeoning barbecue fuel market, Husky Oil in early 1959 acquired the Dickinson Briquetting Company of Dickinson, North Dakota and formulated plans for manufacturing briquets from nearby lignite coal beds. When preliminary tests of the new lignite briquet showed poor odor and ignition characterisitcs, Husky retained Arthur D. Little, Inc. to correct these faults and develop a process and program for producing an improved and competitive lignite briquet.

Though Husky had hoped to enter the market with its barbecue briquet in 1960, it semed advisable to postpone entry until after a

comprehensive research and development program had been completed. The program which ADL recommended included carbonization studies, ignition studies, odor studies, pilot plant testing, plant design and construction, and plant operation.

The overall objective of the program was to produce a barbecue briquet from lignitic coal which would have properties equivalent or superior to those of successful charcoal briquets already on the market.

Essentially, the approach to this objective was to analyze the problems involved, to find reasonable solutions for them, and to supply those solutions to the design, construction and operation of a commercial facility. A team composed of Husky and ADL personnel, representing various disciplines, was organized and the first phase of the program—a series of carbonization studies—was begun in January 1960.

Simultaneously, with the carbonization studies, work was also begun on odor and ignition problems. In the carbonization studies the team examined various carbonized samples and was able to: (1) establish operating standards for the carbonizers; (2) demonstrate reproducibility of operation. Through the odor project the team was able to identify, isolate and eliminate all of the objectionable aromas associated with earlier briquet samples. The odor elimination was accomplished by substituting a clean, inert gas for recycled gas in the carbonizers, by controlling the carbonizer temperatures, and by removing the sulfur-laden pyrite mixed in with the lignite.

Our ignition studies involved selecting the correct kind and amount of binder for holding the char and other ingredients of the briquet together. Consideration was also given to promoters and oxidants to make ignition easier. The Husky-ADL research team decided against using promoters or oxidants, and by properly selecting size distribution and concentration of the binder particles, a briquet was produced with excellent ignition and odor characteristics.

To confirm the laboratory results, obtain process and plant design data and provide sample briquets for consumer testing, a pilot plant program at the Dickinson facilitiy was initiated in June 1960. Pilot plant production was rated at 200 pounds per hour; the briquets produced were tested and found comparable to those made in the laboratory. One ton of briquets was packaged in Dickinson and sent to ADL's Cambridge facilities for consumer testing. Test results showed good acceptability and at the same time indicated several ways in which the product could be improved.

In view of generally favorable reaction to the briquets, and after making the changes indicated, the Husky-ADL team designed a commercial plant whose production capacity would be about six tons of briquets per hour. The steps in the commercial process, which were identical to those of the pilot runs, consist of carbonization, pyrite separation, grinding, mixing, briquetting, drying and pack-

aging. Equipment was selected on the basis of performance during pilot operation or on the basis of vendors' performance specifications. Plant construction began in the Fall of 1960 and was completed in March 1961. Full production commenced in June 1961.

Research was then concentrated on the liquid side of the carbonization operation to determine other types of products which could be produced from lignite.

Products Contemplated

Two types of products can result from low-temperature carbonization of lignite-solid-derived and liquid-derived. On the solid side, consideration was given to metallurgical coke, foundry coke, activated carbon and further production of fuel briquets— the product of the Dickinson facility for over 25 years. For the present and immediate short-term future, the only solid product that is being contemplated is the barbecue briquet.

On the other hand, the liquid-derived products are many and it is proposed that some or all will be produced in the not-too-distant future. These are pitch, creosote, phenols, cresols, xylenols, guaiacols, catechols, napthols, quinolines, pyridines, analines, and neutral oils— intermediate products for use in plastics, weed killers, disinfectants, flotation oils, fuels, timber preservatives and rubber extenders to mention a few end-usages.

Present Operation and Products

Let us now look in detail at the present operating facility in Dickinson, North Dakota to see how the plant operates. As you will recall from the earlier discussion, the facility, as far as solids are concerned, follows the processing steps proven out during the pilot operation—carbonization, pyrite separation, grinding, mixing, briquetting, drying and bagging.

Lignite is mined from an open pit near the plant and delivered to the plant by truck. It is fed to a crusher and is reduced in size to approximately four inches and smaller. The crusher is protected by a magnetic pulley for elimination of tramp iron.

The crushed lignite is lifted by bucket elevator and passed over a vibrating screen with the undersize going to the power plant. The screen has ½ inch square openings but the size can be changed easily to meet the needs of the power plant. Combustion of the undersize furnishes the heat and part of the power required for the operation of the carbonizer, power plant and the briquet plant. The balance of the power is purchased from the R. E. A.

The oversized lignite from the screen is stored in three large coal storage bins. The lignite is then fed into the bunkers of two Lurgi carbonizers. The basic idea of the carbonizers is to subject the coal to circulation of gases. The heat is transferred directly to the coal by direct contact of the gases with the coal. The carbonizer retort has two main parts: the upper or predryer section where the 39%

moisture in the lignite is removed; and the lower or carbonizing section.

In the predryer section, the lignite is dried by the gas that is produced from the carbonizer section, which is mixed with air and burned outside of the predryer section. The coal is distintegrated in this section so that a product ¼ inch to ¾ inch is delivered to the carbonizing section. The dried lignite is delivered by means of gravity flow through eight connecting chutes to the carbonizing sections. The chutes also act as a seal between the predryer and carbonizing sections. This type of arrangement eleminates any additional cost of handling the coal besides eliminating the cost of additional equipment.

The gas pressure in the predryer is a few millimeters greater than the pressure in the carbonizing section and due to this pressure differential, as well as the resistance in the chutes, which are full of lignite, no gas flow results from the carbonizing section to the predrying section. Thus, there is no risk of combustion in the predrying section. There are no valves or other obstructions in the chutes to interfere with the flow of coal through them.

The carbonizing section is run on the same principle as the predrying section with similar forms and ducts. As the coal reaches the carbonizing temperature, the tar is driven from the coal leaving a char, composed of fixed carbon, ash and volatile matter. The char gravitates to the bottom of the carbonizer where it is cooled by entrant cooling gas. This gas does not burn in this section because of the absence of air. The gas and the tar from the lignite are drawn off the top of the carbonizing section and sent to the liquid side of the plant for tar and creosote recovery. The cooled char is removed at the bottom of the carbonizing section by an air-lock valve system. The char then passes over a bar screen to remove the iron pyrites that did not disintegrate during carbonization, and is then crushed in a hammermill to approximately 4 mesh and stored in a 100 ton hopper.

During the fall and early winter months, fuel briquets are produced. For this product, the char is passed by means of a table feeder to a long paddle mixer, where pitch and asphalt binder are mixed with the char. A predetermined amount of water is mixed with the char and binder before it is conveyed to a vertical fluxer. The fluxer empties into a mixer that feds a Komarek Greaves briquet press.

The briquet press will briquet the wet mix at a rate of 18 tons per hour. The green briquets are discharged to a cooling belt that conveys them to a cooling storage bin. The green briquets are cooled for 24 hours when they can be loaded into box cars in bulk or can be stored in piles in the open.

During the balance of the year the char is made into barbecue briquets. Prior to briquetting, the char is passed by means of a table feeder and Barber-Greene belt conveyor to two Sutton-Steele air flotation tables where most of the remaining iron pyrites are removed. The char from the air flotation table is then conveyed to a Raymond

roller mill where the char is ground to the particle size desired for briquetting. The fine char particles are pneumatically conveyed to a cyclone at a high elevation, with the air returned back to the blower for re-use in conveying the fine char. An electrically driven rotary valve discharges the char from the cyclone into the fiften ton storage bin. The reason for bringing the char to a higher elevation is to allow gravity flow of all material thus eliminating additional handling and equipment costs.

Starch, the binder, is brought to the plant in bulk and is pneumatically conveyed from Aeroslide railroad cars to a bulk storage tank. The starch is then pneumatically conveyed from the storage tank to a smaller storage bin located at the processing line.

In mixing the char for briquetting, a predetermined amount of char and starch binder are weighed by two Toledo scales into a char and starch weigh hopper. The two weigh hoppers discharge into a large Simpson Mix-Muller where the binder and char are thoroughly mixed dry. Then a predetermined amount of water is added to the dry mix by a bowser water meter. At the end of the mixing cycle, the Muller discharges the wet mix to a Strong-Scott ribbon blender that feeds two Komarek-Greaves briquet presses.

All of the weighing, mixing and discharging of the mix is operated by a General Electric timer that controls all opening and closing of valves. While the mixing cycle is on, the Toledo scales are weighing a new batch of char and binder for the next mix. This batch is readied for discharge to the Muller after the Muller has discharged the previous batch.

The two presses briquet the wet mix at a rate of three tons per hour per press. The green briquets are discharged onto a belt that conveys them to a roller screen where the fines are screened and returned to the blender by means of a Redler conveyor. The screened briquets are then conveyed to an oscillating conveyor that distributes the green briquets evenly across another conveyor in a Proctor & Schwartz steam dryer.

The green briquets are conditioned and heated up in the heating section of the dryer for a sufficient length of time to reduce the moisture in the briquet to approximately 4%. In the last section of the dryer, the briquets are cooled before being discharged.

The dried briquets are screened and conveyed to five cooling bins where they are allowed to cool for 72 hours. The dried cooled briquets are then packaged with two Aeroglide Weigh Packers into 5, 10, 20 and 40 pound "Grill Time" and "Husky" bags, to be stored in two 160 ft. x 200 ft. warehouses or loaded into box cars. Siding space is available for the loading of eight railroad box cars at one time.

In conjunction with both operations the tar that is recovered from the retorts is distilled in a batch type distillation unit. The tar is heated in a still with the creosote fraction being vaporized. The

creosote is cooled and recovered in several receivers. It is then pumped to storage where later it is sold as a wood preservative to the railroad companies and allied industries. The residue of the distillation or the pitch is pumped to a storage tank where it is then used as a binder for fuel briquets.

Marketing

During the past few years the gradual increased distribution of natural gas and burner fuel has prompted many users of fuel briquets to convert their heating units to the more desirable fuel. As a result of this, the market for these briquets has been decreasing each year. It is predicted that production of this briquet at the Dickinson facility will be discontinued during the next year.

Unlike the demand for fuel briquets, the demand for barbecue briquets is increasing each year. Outdoor cooking has caught on rapidly throughout the nation creating an increased demand for briquets at a rate of 10 to 15% each year. As a result of this, Husky's interests are directed to this type product for the solid fuel fraction of the operation.

In the first year of operation, the Husky briquet was distributed and sold in four provinces of Canada and 18 states. In 1962, Husky has expanded its distribution using top brokers and nationally known merchandising firms across the nation and the Husky briquet is available in five provinces and 36 states.

As has been mentioned previously, considerable interest is being directed toward the liquid by-products that can be produced from a low temperature lignite coal tar. The properties of the tar are such that it is not difficult to handle on a commercial scale, giving products of interest to both the chemical and the liquid fuel industries. As the present research program is continuing, marketing and sales plans are being considered. In the not-too-distant future, other processing lines will be in Dickinson to produce some of the products mentioned earlier.

CONCLUSION

The uses of lignite and its by-products are under continuous study by the Husky-ADL research team. This new area of activity presents both challenge and promise for Husky's future. The plant operating on one of this country's largest solid fuel deposits on the rolling plains of North Dakota is now manufacturing certain products and is researching others for eventual nation-wide distribution in the United States and Canada.

---

[1]Plant Manager, Husky Briquetting Division, Husky, Oil Company.
[2]Senior Project Engineer, Arthur D. Little, Inc.

KINETICS OF THE KETO-ENOL TAUTOMERIZATION OF ACETOACETALDEHYDE AND OF ITS CONDENSATION-POLYMERIZATION TO TRIACETYL BENZENE

Franz H. Rathmann and Irene Swanson

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

ABSTRACT

In previous papers (2) (3) it has been shown that acetoacetaldehyde (I) and hydroxy-methylene-acetone (I) exist together in a rapidly established tautomeric equilibrium, and that the ionization constant of the enol form (I) as an acid is about 1×10^{-6}.

Further studies on the course of the change in the pH of solutions of the sodium salt of acetoacetaldehyde when 10-20 mole percent of acid is needed, or of solutions of the free keto-enol equilibrium mixture when a base is added, show that the first-order half-life of the enol form is of the order of eight seconds at room temperature (1) (4). At 0.100 molar, the first order enol———→keto reaction is almost overshadowed by the rapid and concurrent second order non-reversible condensation-polymerization reaction to give triacetyl-benzene (III).

At 0.01 to 0.04N, the second order reaction is relatively slow, and it is much easier to distinguish between the first and second order components of the concurrent reactions.

BIBLIOGRAPHY

1. Creighton, H. Germain, "Principles and Applications of Electrochemistry", John Wiley and Sons, Inc., New York (1935), p. 367.
2. Dahm, Donald, Roger Johnson, and Franz H. Rthmann, "Preparation and Characterization of Acetoacetaldehyde". Proc. No. Dak. Acad. Science XIII, 19-23, (1958).
3. Dam, Lonald, and Franz H. Rathmann, "Titrimetric and pH+ Studies on the Keto-Enol Tautomerism and the Enol Acid + Dissociation of Acetoacetaldehyde (Butanone-3-al-1), Proc. No. Dak. Acad. Science XIII, 37-41, (1959).
4. Glasstone, Samuel, "An Introduction to Electrochemistry". D. Van Nostrand Company, Inc., New York (1942), pp. 410-412.
5. Ingold, Ch. K., "Structure and Mechanism in Organic Chemistry", Cornell Univ. Press, Ithaca, N. Y. (1953) pp. 567-571.

CHLOROMETHYL ZINC

Virgil I. Stenberg and Arlan D. Norman[1]

Department of Chemistry

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

With the recent interest in reactions involving divalent carbon intermediates, attention has been given to reagents which are precursors to these intermediates. Of current vintage is $IZnCH_2I$ which is prepared by the reaction of methylene iodide with a zinc-copper couple. This procedure has not been successful with methylene chloride.

Our work on the reactions of alpha-diazo ketones led to the discovery that zinc chloride reacts with diazomethane liberating nitrogen. The product is relatively unstable. On exposure to moisture, a white cloudiness forms immediately with the simultaneous liberation of the gas, methyl chloride. Attempts to distill the product lead to decomposition to an amorphous white material believed to be polymethylene.

The chemical reactions and spectra of the product provide an insight into the structure of the zinc compound. Infrared analysis indicates the presence of carbon-hydrogen bonds. The compound is unable to undergo the normal carbanion reactions of the Grignard reagent and dimethyl cadmium which points to a minimum contribution of an ionic zinc-carbon bond. Further, its reaction with acetyl chloride and chlorine to form colored intermediates before decomposing to products, the formation of polymethylene, and the reaction with cyclohexene instill the concept of a zinc chloride-methylene complex.

[1]Senior Honors Program Student.

REACTION OF TITANIUM TETRACHLORIDE WITH SOME ALCOHOLS

D. Schwartz, B. M. Morgan, W. D. Cross and A. E. Rheineck

College of Chemical Technology

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

ABSTRACT

In 1875 Demarcay reported the reaction of three moles of ethanol with one mole of titanium tetrachloride (2). A crystalline substance was obtained which appeared to be tetraethoxy-titanium. Bischoff

and Adkins (1) later showed the compound to be diethoxy-dichloro-titanium and not the tetra substituted compound, which is a clear liquid. However, Demarçay is given credit for the first synthesis of an organo-titanium compound.

Since their discovery, organo-titanium compounds have been used extensively as catalysts for polymerization reactions (3, 4, 6), as modifying agents for resins and have been used as vehicles in paints. A lesser amount of work has been done on incorporating titanium into polymers (5).

In the present study, titanium tetrachloride was treated with various straight chain alcohols containing from 2-10 carbon atoms. The products appear to be dialkoxy-dichloro-titanium. These compounds are crosslinking agents in the preparation of polymers from epoxides. The film properties of these titanium containing copolymers have been evaluated.

BIBLIOGRAPHY

1. Bischoff, F. and Adkins, H., J. Am. Chem. Soc., **46,** 256-7 (1924).
2. Demarcay, E., Comptes Rendus, **80,** 51-3 (1875).
3. Jezl, J. L., U. S. 2,935,496.
   C. A. **54,** 17964 (1960).
4. Kabanov, V. A., Zubov, V. P., and Kargin, V. A., Vysokomolekuyarnye Socdineniya, **1,** 1422-7 (1959).
   C. A. **54,** 17951 (1960).
5. Taylor, J., J. of the Oil and Colour Chemists' Assn., **43,** 555-64 (1960).
6. Young, D. W. and Kellog, H. B., U. S. 2,446,897.
   C. A. **42,** 7574 (1948).

PHOTOMICROSCOPY AND THIXOTROPIC BEHAVIOR OF SOME TOLYL URETHANES

Jon A. Griepentrog and Sol Shulman

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

College of Chemical Technology

ABSTRACT

The effectiveness of long chain aliphatic urethanes (carbamates) in imparting thixotropy or gel-forming properties to a variety of materials has been reported previously (1). The application of chemical microscopy to the correlation of crystal structure and thixotropic be-

havior of aliphatic urethanes has been the subject of another study (2).

This paper reports the thixotropic behavior of tolyl urethanes prepared from aliphatic alcohols (containing from 1 to 22 carbon atoms) and **o-**, **m-**, and **p**-tolyl isocyanates.

These urethanes give thixotropic gels with hydrocarbons (e.g., petroleum ether) and other relatively nonpolar materials. Urethanes derived from long chain fatty alcohols (e.g., octadecyl N-**o**-tolyl-carbamate) impart thixotropy to more polar materials, such as ethanol.

In general, the urethanes derived from primary straight-chain alcohols show better thixotropic properties than those derived from branched and/or secondary, or tertiary alcohols. In addition, the following generalizations may be made. The **o-tolyl** urethanes show the most effective thixotropic properties. The **p**-tolyl carbamates were next in effectiveness, and the **m-tolyl** derivatives least. In the **o**-tolyl series the thixotropic properties (in petroleum ether) decrease gradually from the methyl derivative to the **n**-butyl **o**-tolylcarbamate. The thixotropic properties increase gradually with an increase in chain length of the O-alkyl group. In the **p**-tolyl series the derivative of 1-butanol is the lowest weight compound to show thixotropic properties, and these properties increase with an increase in chain length of the O-alkyl group. In the **m**-tolyl series the derivative of 1-decanol is the lowest weight compound to show thixotropic properties. In this case the thixotropic behavior improves also with an increase in chain length of the O-alkyl group.

Photomicrographs of these compounds have been prepared and it was noted that tolyl urethanes showing thixotropic characteristics have fine needle or cluster of needle type structures. Compounds showing no thixotropic properties have rod-like crystal structures. This observation is in agreement with previous work on aliphatic urethanes (2) and phenyl and naphthyl urethanes (3).

In addition, the photomicropgraphs of these derivatives (and their melting points) may be used as an aid in the characterization of alcohols.

REFERENCES

1. S. Shulman, M. W. Formo, and A. E. Rheineck, **J. Amer. Oil Chemists' Soc. 38,** 205 (1961).

2. R. C. Hendrickson and S. Shulman, **Microchem. J. 5,** 587 (1961).

3. S. Shulman, C. A. Knutson, Jr., and F. L. Minnear, in press.

KINETICS OF THE SLOW THERMAL DECOMPOSITION OF SIMPLE NITRATE ESTERS AND OF POLY-NITRATE ESTER EXPLOSIVES[1]

Franz H. Rathmann

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

As shown by Roginski (5), the slow thermal decomposition of nitrate ester explosives exhibits a reaction rate that differs markedly from that of the simple nitrate esters. These simple esters decompose with an activation energy of 39 and 37 kcal. per mole, and have a frequency factor characteristic of 10^{14} to 10^{16} for first order reactions. In general, the decomposition kinetics of the polynitrate ester explosives require activation energies of up to 50 kcal. These reactions appear to proceed with frequency factors up to 10^{23} or even 10^{25}.

Careful studies using a variety of methods have been carried out on the slow thermal decomposition of polyvinyl nitrate (PVN) from 100° to 170° C, as well as on a number of mono- and dinitrates esters at temperatures from 100° to 250° C.

The thermal decomposition of PVN in methyl cellosolve solution was carried out at 105° and 120° C. Measurements were made on the pH of the solution, the concentration of nitrate ester and of carbonyl group, and on the viscosities of the solutions as functions of time and extent of decomposition. The nitrate ester data indicate an energy of activation or E value of 38 kcal per mole, whereas the viscosity data give an E value of 42 kcal per mole. Similar data for decomposition in cyclohexane at 120° to 150° indicate an E value of 45 kcal per mole. Viscosity data on the decomposition in adiponitrile solution at 100°-120° give an E of 33 to 36 kcal per mole, and a frequency factor value A, of 10^{10} to 10^{13}.

For decomposition in the undiluted bulk state at 140°-150°, E is 45-50 kcal per mole. The same value is obtained on heating thin films **in vacuo** on a quartz spiral balance.

Burning rate studies (1) (2) at nitrogen pressures from 100 to 35,000 lb. per in.^{2} show good agreement with the Muraour equation (3) (4);

$$r = a + bP,$$

with a = 0.20 m/sec., b = 1.8×10^{-4} lb./in. sec. The themodynamically calculated temperature of combustion obtained from the frozen CO-CO_2-H_2-H_2O equilibrium is 1,200 to 1,240° K.

The thermal decomposition of simple nitrate esters in the early

[1]This paper is a summary of the as yet unpublished work done by the author and numerous colleagues under the general direction of Bryce L. Crawford, Jr., as part of Naval Ordnance Projects No. 10345 at the University of Minnesota. The details of the work will be published elsewhere.

stages of decomposition proceeds according to first order reaction kinetics, $-k = Ae^{-E/RT}$; E is approximately equal to 40 kcal per mole for all nitrate esters studied, and A is of the order 10^{12} to 10^{16} molecules per mole per second.

Various nitrate esters were vaporized in a stream of nitrogen and passed through a furnace at temperatures from 200° to 260° C. The products were analyzed for carbonyl content, NO_2, formaldehyde, and acids. From these data values were calculated for E and A.

Decompositions were also carried out in cyclohexane solution at 120°, 140°, and 160° C. The E and the $Ax10^{-14}$ values obtained were: sec. butyl, 28.4, 1.4; n-amyl 39.3, 2.5; cyclopentyl 36.4, 3.4; capryl, 37.3, 4.7; decyl 41, 57; decyl (in methyl cellosolve) 31, 51; decyl in adiponitrile 38.5, 7; α-chloropropyl, 43, 4; 1, 3-dinitroxy butane 43, -; 2, 3-dinitroxy butane, 41.0, 2 x 10^3; 2,4-dinitroxy-pentane, 39, 10^3; polyvinyl nitrate 40-45, 10^4. For primary nitrates we have $k_1 = 1 \times 10^{-1}$, and for secondary nitrate $k_1 = 2 \times 10^{-1}$ and for disecondary nitrates, $k_1 = 8 \times 10^{-1}$. The dinitrates exhibit high values of A.

Mass spectroscopic evidence indicates fragmentation is primarily to NO_2, CH_2O, and alkyl radicals.

$$R\text{-}CH_2\,ONO_2 \longrightarrow R + CH_2O + NO_2$$

Actual explosvies contain added inhibitors to decrease autocatalytic decomposition. Unfortunately the inhibitors also enter into a second-order reaction with the nitrate ester. The total kinetics is then given by a differential equation involving one first-order and three second-order rate constants. Experimental data on the decomposition of decyl nitrate with from zero to two moles of diphenylurea, at temperatures from 100° to 170° C, indicate the correctness of this four-constant kinetics of reaction theory.

REFERENCES

1. Crawford, Bryce L., Jr., Clayton Huggett, Farrington Daniels, and R. E. Wilfong, Direct Determination of the Burning Rates of Propellant Powders, Analytical Chemistry 19, 630-3 (1947).
2. Crawford, Bryce L., Jr., and David Rotenberg, Polyvinyl Nitrate Strand Burning-Rate Measurements in the Minnesota Electrical Bomb
3. Muraour, Henri, and Jean Fauveau, Laws of Combustion of Colloidal Powders in Nitrogen Gas Under Pressures from 1,000 to 10,000 kg./cm^2. Compt Rend. Acad. Sciences Paris 231, 1043-5 (1950); also Chemie et Industrie 65, 53-5 (1931).
4. Pekkarinen, Errki, Burning of Powders, Soc. Scient. Fennica, Com. Phys. Math. XIII, No. 10, 1-114 (1947).
5. Roginski, S. Z., Decomposition of Explosives at Low Temperatures, Physikalische Zeitschrift der Sowjet Union 7, No. 5, 640-699 (1932).

WRINKLING PHENOMENON AND MECHANISM OF THERMAL POLYMERIZATION OF TUNG OIL: A PRELIMINARY REPORT

A. E. Rheineck and S. Chung Suen Peng
Coatings Technology Department
North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

ABSTRACT

Films of heat-bodied tung oil do not wrinkle on drying while films of raw tung oil wrinkle.

Thermally treated tung oil was subjected to oxidative degradation and it was found that the polymerized oil consists of groups of eleostearic acid radicals.

In addition, the presence of conjugated dienoic acid was shown using ultra-violet spectrophotometric methods. It is assumed that trimmers and higher polymers were also formed, but their presence has not yet been confirmed.

It was concluded that the conjugated triene system was responsible for the wrinkling phenomenon.

The structural changes of tung oil during air drying were also investigated. Using ultra-violet spectrophotometric methods, it was shown that a conjugated diene system was formed at the expense of conjugated triene during oxidation. Infra-red spectrophotometric studies during air drying indicated the formation of a peroxide which was converted (by further air oxidation) to a ketone. Polymerization took place during oxidation and showed a decrease of **trans**-and **cis**-carbon-carbon double bonds.

Wrinkling of tung oil films can be eliminated by "blocking" the conjugated triene system either by (a) air blowing, (b) epoxidation, or (c) introduction of oxidation rate controlling agents (e.g., 1, 10-phenanthroline) into the oil. These treatments apparently cause the upper surface and the inner layer of the film to dry at equal rates.

DIMORPHOTHECA OIL: SOME FILM FORMING CHARACTERISTICS

A. E. Rheineck and H. Sobol
Coatings Technology Department
North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

ABSTRACT

Dimorphotheca oil (1) is obtained from the seeds of **Dimor-**

photheca Aurantiaca (Cape Marigold), family of Compositae. The main component is a 9-hydroxy-10, 12-octadecadienoic acid, named "dimorphecolic acid" (2).

The raw oil dries with difficulty in air. Wrinkled films form after about 40 hours. The allylic structure of 9-hydroxy-10, 12-octadecadienoic acid appears to be responsible for the inhibition of air drying of the raw oil.

Heat-bodied oil dries within several hours. Chemical changes taking place during heat bodying were observed with the aid of ultra-violet and infra-red spectrophotometry. It was noted that the predominating reactions and the ultimate products are dependent on the temperature of reaction.

Thus, when the oil is heated at 200°C., dehydration and polymerization takes place at about the same rate. When the temperature is raised to 280°C. the rate of polymerization is greater than the rate of dehydration. In addition, the higher temperature seems to favor the formation of a cyclic polymer.

Dimorphotheca oil can be dehydrated by means similar to those used for dehydration of castor oil (e.g., 3% $KHSO_4$, 90 min., at 115°C). The dehydrated oil dries rapidly.

Phenolic resin varnishes of Dimorphotheca oil are essentially equivalent to similar varnishes based on tung oil.

REFERENCES

1. Earle, F. R. and Wolff, I. A., J. Amer. Oil Chemists' Soc. 37, 254 (1960).
2. Smith, Jr., C. R., Wilson, T. L., Melvin, E. H., and Wolff, I, A., J. Amer. Chem. Soc. 82, 1417 (1960).

IMPROVEMENTS IN FILM-FORMING PROPERTIES OF LINSEED OIL

A. E. Rheineck, F. D. Williamson and D. deClerck

Coatings Technology Department

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

ABSTRACT

Although the coatings industry has been enjoying a period of vigorous growth, the consumption of linseed oil has been steadily declining. A major factor for this decline are several inherent weaknesses of linseed oil which limit its use in coatings. Some of these weaknesses are gloss retention, hardness, yellowing and poor chemical resistance of films. In this study we wish to report some pre-

liminary results in an attempt to improve the film forming properties of linseed oil.

Linseed oil was partially epoxidized using the resin-bed technique (2) to an oxirane content of 3.0-3.5%. The partially epoxidized linseed oil had about the same amount of unsaturation as soybean oil, thus permitting drying by air oxidation. It was shown with the aid of ultra-violet spectrophotometry that while the dienoic acid content remained essentially constant before and after epoxidation, the trienoic acid content decreased to a very low level. At the same time, the tetraenoic acid content showed a correspondingly large increase. It is speculated that this increase in tetraenoic acid content may be due to selective epoxidation of the linolenic acid with resulting dehydration and/or deacylation of the alkoxy-hydroxy compounds formed during the epoxidation process.

Based on oxidative degradation studies using liquid-liquid partition chromatography methods (1) it was shown that the 12 carbon-carbon double bond of linolenic (**cis**-9, **cis**-12, **cis**-15-octadecatrienoic) acid was preferertially epoxidized. This selective epoxidation is favored when the resin (Dowex 50 x 8) H_2O_2 ratio is 0.585.

The partially epoxidized linseed oil was cross-linked with various polyfunctional acid anhydrides and with boron trifluoride in an attempt to improve the film-forming characteristics of the oil. It was found that partially epoxidized linseed oils cured with boron trifluoride exhibited exceptionally good film forming characteristics.

REFERENCES

1. Corbin, E. A., Schwartz, D. P., and Keeney, M., J. Chromatog. 3, 322 (1960).
2. Schmidtz, W. R. and Wallace, J. G., J. Amer. Oil Chemists' Soc. 31, 363 (1954).

MICROLITHOLOGY OF A SECTION IN UPPER GLACIAL LAKE AGASSIZ SEDIMENTS AT GRAND FORKS, NORTH DAKOTA

S. J. Tuthill, Lee Clayton, and G. G. Thompson

Department of Geology

University of North Dakota, Grand Forks, North Dakota

INTRODUCTION

The excavation of a sewer pipe ditch along the south side of the west end of Sixth Avenue North, Grand Forks, North Dakota, in the spring of 1961, provided an opportunity to inspect and sample

the upper portion of the sediment of glacial Lake Agassiz. The pipe laying was a continuous operation; the ditch was dug, pipe laid, and the excavation filled without interruption. This did not provide optimum conditions for sampling and inspection, but the sample was taken with only one lateral shift of sampling site. A 20 inch section in the upper part (821.6 to 823.2 ft. elevation, 3.3 to 4.9 ft. depth) of the ditch was not sampled because of disturbance of the sediments by the digging equipment. The samples were inspected under a binocular microscope at a magnification of x45. Five parameters were chosen as variables and their vertical distribution was recorded. These were: grain size of the sediments, color, secondary minerals, stratificaion, and fossil content.

PREVIOUS WORK

The earliest comprehensive study of glacial Lake Agassiz was by Upham in 1895. Theoretical histories of the lake have been postulated by Upham (1895), Tyrell (1896), Leverett (1913 and 1932), Johnston (1916, 1917, and 1946), and Elson (1957a and 1957b). All have been based primarily on geomorphic evidence and organic sediments at various places along the margin of the lake sediments but not the lithologic character of the sediments as such except where they are exposed at the beaches. Dennis and others (1949) and Romiger and Rutledge (1952) studied the lithologies of core samples taken from Lake Agassiz sediments. Their purposes (ground water survey and a lithologic study respectively), and therefore their approaches, were different. Dennis and others logged the gross lithology of 73 wells as described in part by them and in part by drillers. They separated Lake Agassiz sediments into two units based supposedly on lithologic criteria. Rutledge and Romiger, on the other hand, subjected core samples to various standard soil mechanics tests. In the light of the results from these tests, they suggested criteria for separating the Lake Agasiz sediments into five units.

Dennis and others state "the silt unit of the Lake Agassiz deposits is the surface rock throughout the area and rests disconformably upon the clay unit of the Lake Agassiz deposits. It is composed primarily of silt, buff to yellow to gray in color, but locally contains sand or clay." They also say, "as the name implies, it is composed primarily of silt . . ., but locally it contains sand and clay. In a few places the entire unit is composed of clay." An inspection of the 76 well logs included in their report leads to a different conclusion. In 61 of the logs, the Lake Agassiz sediments have been divided into two units. Of these, 29 list clay as the only lithology of the "silt unit" and only three list silt as the only lithology. Of the remaining 15 logs, 12 list clay as the only lithology for the combined "silt" and "clay" units. Forty-one of the 61 wells that have the "clay" and "silt" units separately logged list yellow, brown, or buff as the color of the "silt" unit and blue, gray, or dark for the "clay unit."

In view of the discrepancy between their text and their data, it seems reasonable that Dennis and others picked the break between the "silt" unit and the "clay" unit at the color change rather than at the change in grain size. Since the color change in the upper Lake Agassiz sediments is very likely the result of penecontemporaneous or post-depositional oxidation of iron compounds, it cannot be regarded as a criterion for detection of a change in the depositional environment or as a basis for separating stratigraphic units. Dennis and others offer no evidence to support their statement that the "silt" unit disconformably overlies the "clay" unit.

Romiger and Rutledge analyzed cored samples of the sediments from the surface of the uppermost till from Fargo and Grand Forks, North Dakota, and Crookston, Minnesota. They subjected the samples to several tests commonly used by civil engineers for evaluating foundation requirements. The most significant test, so far as this discussion is concerned, is the preconsolidation stress test. This consists of measuring the volume reduction in a confined clay sample when it is subjected to a standard stress. From the curve of stress versus decrease in volume, the degree of compaction of the clay is established. Assuming previous load to be the critical causative force for any compaction value less than the ideal laboratory value, Romiger and Rutledge postulate that the attractive capillary force associated with a dessication surface could also effect the compaction value of clay. Thus they suggest an unconformity between the upper Lake Agassiz sediments and the lower ones. Unfortunately they do not present preconsolidation stress values for the Fargo area, thus making impossible a comparison between the well logs reported by Dennis and others and Romiger and Rutledges' inferred stratigraphic units for that area.

Elson (1957a) suggests the terms "Lake Agassiz I" for the lower unit, which is typified by dark fat clays and "Lake Agassiz I" for the upper yellow or tan silty unit. Unfortunately Elson's detailed studies have never been published, but in the 1957a article, which is a resume of a more complete work, he suggests lithology as the criterion by which the two units are distinguished. Elson studied geomorphic aspects of the Lake Agassiz sediments and is involved in the problem of explaining the manner and sequence of beach formation. His terms "Lake Agassiz I" and "Lake Agassiz II" have taken on a stratigraphic implication, however, and must be evaluated in any stratigraphic consideration of the upper sediments in the Lake Agassiz basin.

METHODS

In April of 1961 we cut a 2-inch triangular column from the north wall of a sewer pipe ditch being dug on the south side of Sixth Avenue in the western outskirts of Grand Forks in northeastern (sec. 6, T. 151 N., R. 50 W.) North Dakota. The sampling was

begun at the base of the ditch at a point 225 feet east of the intersection of Sixth Street North and Stanford Avenue. One 20-inch section at the 821.6 to 823.2-foot level was not sampled because the treads of the digging equipment had smeared the exposure. Each sample was measured, cut from the wall, and immediately wrapped in a plastic film sheet. A label was included in each package, and the orientation and sequence was thus preserved. The samples were stored in the original wrapping until the winter of 1961-62. In all cases the samples remained moist and apparently unchanged.

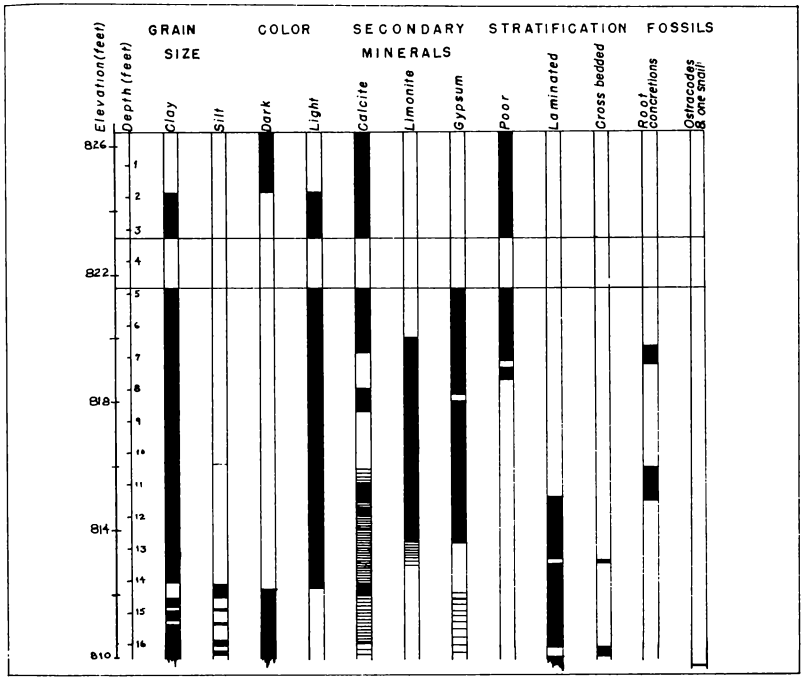


Figure 1.—LITHOLOGIC VARIATION WITH DEPTH. The darkened portion of each column indicates the presence of the parameter. The section between 3.2 and 4.9 foot depth was not sampled because of contamination of the outcrop.

The samples were examined under a binocular microscope (magnification x45), a continuous surface was reacted with dilute HCl and the variations in euhedral calcite and gypsum, limonitic stain, grain size, bedding, fossil content, and color were logged. These variations are shown with depth in figure 1.

Using a slightly modified form of the American Society for Testing Materials hydrometer analysis procedure D422-54T (1958),

analyses were made on a portion of each sample to establish median grain size distributions. Figure 2 shows these results.

RESULTS

The character of the samples is easily seen in figure 1 and 2. The calcite is in the form of minute euhedral dogtooth spar crystals 0.1 mm or smaller in greatest dimension. It occupies the more porous sections of the sediments and gives them a granular or silty appearance. Much of the yellow (iron oxide stained) upper portion of the sediments is rich in these crystals and the actual grain size of the sediments is thus masked by the secondary calcite.

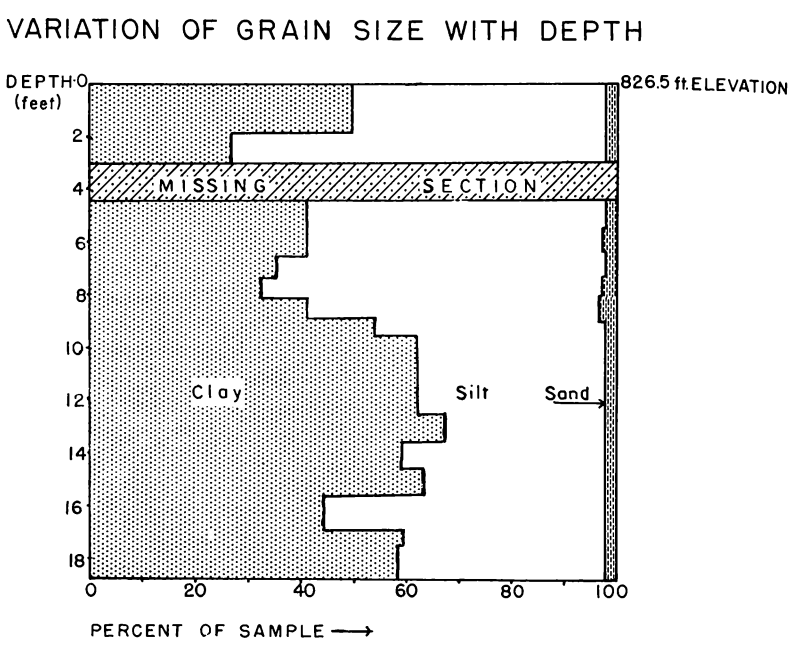


Figure 2.—VARIATION OF GRAIN SIZE WITH DEPTH. The grain size distribution of fifteen samples was evaluated by ASTM hydrometer analysis. The variation with depth is shown in percent of the whole sample.

Gypsum crystals in the form of blades and rosettes 1.0 mm or smaller are very common throughout the middle part of the section. Near the 815 level (13-14 feet below the surface) the calcite and gypsum crystals are concentrated in alternating laminations. Like the calcite, the gypsum is secondary.

Near the surface, limonite is disseminated throughout the sediments and cannot be detected as discrete particles. Limonite is

locally concentrated in two ways in the section. In the middle of the section (820 to 814 foot level, 6.5 to 12.5 foot depth) particles of limonite can be distinguished at the magnification employed. In areas where roots formerly penetrated the soil, limonite concretions are observed. The structure of the plants' roots is commonly observable, and some of the voids left by the decay of the plant material are filled with calcite or gypsum crystals. As these concretions are oriented vertically and bifurcate in a manner which suggests a life position, they may be an important consideration in interpreting the lake's history. They occur at the 819 and 816 foot level (approximately 11 and 7 foot depths respectively). These concretion zones are separate from each other and not connected with the surface. Therefore it is suggested that they may prove to be criteria for reconstructing depth values of the lake.

Bedding varies from massive to thinly laminated. The laminations have been explained previously by a process akin to that by which varves are formed (that is, frozen and thawed condition of the surface of the lake determines grain size of sediments). Overturn phenomena explains this type of rythmic sedimentation equally well and are further favored by the chemical nature of the secondary minerals which occupy the lamination parts. If the lake lacked putresibles, the gypsum layers may represent the deposition during the period of overturn during which the hypolimnion was in equalibrium with the atmosphere, thus reducing the amount of calcium carbonate which would precipitate at depth. When the lake became stratified calcite would precipitate because of the reduction of carbon dioixide pressure in the hypolimnion.

The hydrometer analysis shows that grain size cannot be used to divide the sediments into two lithologic units as described by previous authors. The 8½ foot level (see figure 2) marks the change from silt to clay. This quantitative data does not agree in detail with the first two columns in figure 1 for two reasons. First, the problem of distinguishing the boundary of clay-silt (1/256mm) is a difficult one even with magnifications of x45; it was this reason which prompted the quantitative tests. Second, the larger grains (those greater than 1/16mm), which were not used in the hydrometer analysis, were mostly euhedral gypsum crystals and limonite lumps. This indicates the median grain size was effected by the growth of secondary minerals. During the visual inspection these secondary minerals were not regarded as a basis for determining grain size. The silty portion at the 16 foot level coincides with a 3 inch layer of cross-bedded sediments which grade sharply into the sediments both above and below. No evidence for a change of environment such as a drying surface is seen anywhere in the section.

The presence of animal life at the 809.6 foot level (17 foot depth) is indicated by ostracode carapaces and one worn columella

from a freshwater turbinate snail. The ostracodes are evidently the first microfossils to be reported from Lake Agassiz sediments. They are probably of the genus **Eucandona cf. E. swaini.** The absence of animal fossils from the upper sediments may be a clue to the rate at which the sedidments were deposited. As the sediments are rich in carbonates, leaching of calcareous tests does not seem a plausible explanation for their absence.

DISCUSSION

The criteria for division of the Lake Agassiz sediments as suggested or inferred by Dennis and others, Elson, and Johnston, are not in agreement with the results of the present investigation. The criteria proposed by Romiger and Rutledge, while of interest, and possibly worthy of further testing, are subject to several hazards which cannot be fully evaluated at present. They state that evaluation of the amount of disturbance of the clay by sampling techniques and preservation of the interstitial water is critical to the success of the preconsolidation tests. It seems reasonable that sediments which contain such strong evidence of post depositional change, as the presence of euhedral crystals and a 14 foot zone of oxidation, might be difficult to evaluate by means of the preconsolidation stress test. That this test yields valid data for which it was designed is not questioned, but that sedimentary history can be directly evaluated from it is questioned.

The upper 13.9 feet of tan clay and silt in the section here described can readily be assigned to the Lake Agassiz II sediment unit of Elson on the basis of color. The upper 16 feet can be assigned to Romiger and Rutledge's unit 5 on the basis of stratification and color. No group of criteria adequate to divide the sediments into lithostratigraphic units seems to exist. Thus it is suggested that the idea that glacial Lake Agassiz was drained and then refilled at some time in its history, as suggested by Upham, still remains in doubt, and the techniques employed by Romiger and Rutledge still remain as questionable aids to stratigraphy.

ACKNOWLEDGEMENTS

The writers wish to recognize their debt to Dr. W. M. Laird, State Geologist and Professor of Geology at the University of North Dakota, for suggestions given during the preparation of this report. We are indebted to Mr. Denis Delorme of the University of Alberta who identified the ostracode carapaces.

BIBLIOGRAPHY

American Society for Testing Materials, Nominclature and definitions, standard methods, suggested methods: Philadelphia, 1958.

Dennis, P. E., P. D. Akin, and G. F'. Worts, Jr., Geology and groundwater resources of parts of Cas and Clay Counties, North Da-

kota and Minnesota, North Dakota Geol. Survey Fround-Water Studies No. 11, Minnesota Ground-Water Studies No. 1, 177 p., 1949.

Elson, J. A., History of glacial Lake Agassiz [abs.], Geol. Soc. Am. Bull., v. 68, p. 1721, 1957a.

————; Lake Agassiz and the Mankato-Valders problem, Sci., v. 126, pp. 999-1002, 1957b.

Johnston, W. A., The genesis of Lake Agassiz: a confirmation, Jour. Geology, v. 24, pp. 625-638, 1916.

————, Records of Lake Agassiz, in southeastern Manitoba, and adjacent parts of Ontario, Canada, Geol. Soc. Am. Bull., v. 28, pp. 145-148, 1917.

————, Glacial Lake Agassiz, with special reference to the mode of deformation of the beaches, Canada Geol. Survey Bull. 7, 20 p., 1946.

Leverett, Frank, Early stages and outlets of Lake Agassiz, North Dakota Agri. Coll. Survey, Bien. Rept. 6, p. 17-28, 1913.

————, Quaternary geology of Minnesota and parts of adjacent States, U. S. Geol. Survey, Prof. Paper, 161, 149 pp, 1932.

Romiger, J. R., and P. C. Rutledge, Use of soil mechanics data in correlation and interpretation of Lake Agassiz sediments, Jour. Geology, v. 60, p. 160-180, 1952.

Tyrell, J. B., The genesis of Lake Agassiz, Jour. Geology, v. 4, p. 811-815, 1896.

Upham, Warren, The glacial Lake Agassiz, U. S. Geol. Survey Mon. v. 25, 658 p., 1895.

SEASONAL ABUNDANCE AND DISTRIBUTION OF *PEROMYSCUS MANICULATUS* ON AN EASTERN NORTH DAKOTA FARMSTEAD[1]

Eldon Greij and D. M. Noetzel

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

ABSTRACT

An eastern North Dakota farmstead was livetrapped in order to determine fall and winter distribution differences of the Prairie Deer Mouse **(Peromyscus maniculatus).**

The September trapping indicated that the mice were relatively abundant throughout the entire farmstead but were most abundant in the north, east, and west shelterbelts. They were present in

[1]Supported in part with funds from National Institutes of Health Grant RG 4743.

smaller numbers around the bases of buildings and in grassy areas, found generally in the south-central area of the farmstead.

A garden area located in the east-central portion of the farmstead yielded a comparatively high number of animals in the fall after which the area was plowed. This left the ground barren during the winter and since it showed no visible signs of mouse activity it was not trapped in February.

The winter trapping indicated that the mice were concentrated in the southern region of the farmstead. No mice were captured in the north and west shelterbelts and the catch in the east shelterbelt was much lower than the September trapping. There was a slight increase around the buildings and a very large increase in the grassy areas.

A large weedy area in the northwest corner of the farmstead yielded a moderate number of animals in September but showed a marked reduction in February.

A fenceline (grassy area) bordering the south side of the farmstead yielded a moderate number of mice in September and numbers increased slightly in February.

Fall populations of Prairie Deer Mice were higher and more uniformally distributed than winter populations. Winter populations are lower and appear to be restricted to lesser exposed areas.

CHANGES IN SERUM TRANSAMINASE DURING MIGRATION OF LARVAL *ASCARIS SUUM IN* LABORATORY RABBITS[1]

Carmen Ingebretson and Myron F. Andrews

Department of Veterinary Science

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

INTRODUCTION

In human medicine the transaminases, serum glutamic-oxaloacetic transaminase (SGO-T) and serum glutamic-pyruvic transaminase (SGP-T), have been used in diagnosing and studying cardiac and hepatic diseases. Several workers (3, 4, 6) have shown that in cardiac necrosis the SGO-T level is elevated, but the SGP-T level increases slightly, if at all. In liver disease, upon tissue necrosis, there is a distinct increase in both the SGO-T and SGP-T levels in the blood.

Recent studies have been made on serum transaminase levels in swine following infection with larval swine ascarids (**Ascaris suum**) (1). This study was undertaken to observe the effect of a heavy infection of larval **A. suum** in an unnatural host.

PROCEDURE

Five clinically healthy laboratory rabbits were given a large number (about 30,000) of embryonated ova of swine ascarids (**Ascaris suum**). Five similar, healthy, noninfected rabbits served as controls. Blood samples were taken from the ear vein of the ten rabbits at 12 hour intervals, from 30 hours prior to infection to 210 hours after infection. The SGO-T and SGP-T levels were determined on each sample by the methods of Gabaud et al (2) and Wroblewski and Cabaud (5). Spectrophotometric determinations were made on a Coleman Junior Spectrophotometer. The enzymes were calculated in units per mililiter of serum.

Upon necropsy the liver and lungs were examined for tissue damage. In four of the rabbits numerous surface scars were seen on the liver and severe pathology of the lung was noted. The lungs were cut into 0.5 centimeter strips and placed in a Baerman apparatus so the larvae could be collected and counted. Over 1,000 migrating larvae were collected from each of four of the infected

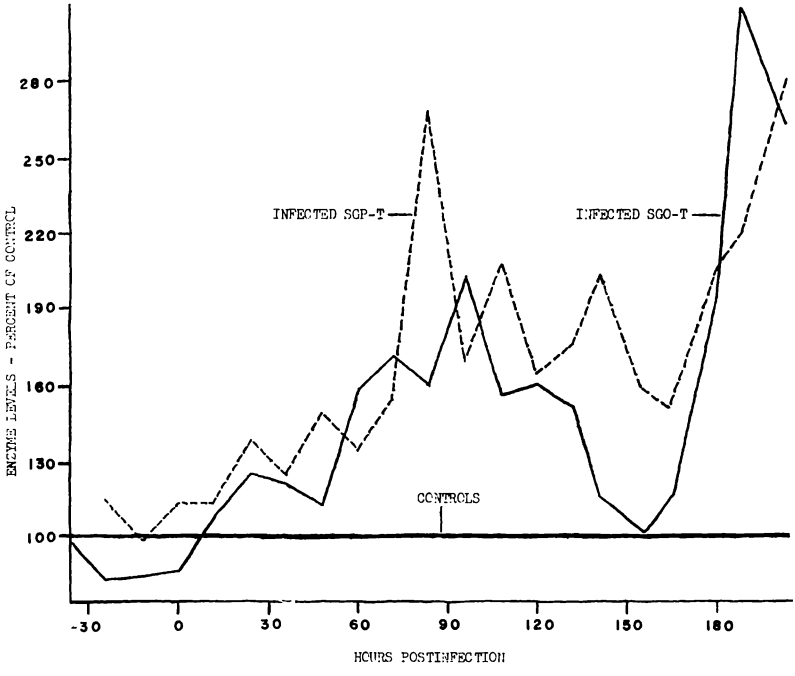


FIGURE 1—Percentage changes inthe serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase levels in rabbits following infection with lethal doses of the larvae of **Ascaris suum.**

rabbits. The fifth infected rabbit was apparently completely resistant to the ascarid infection. No changes were found in the SGO-T and SGP-T concentrations. At necropsy no pathology was evident, and no **A. suum** larvae could be recovered from the lungs. The enzyme levels from this rabbit are not included in the results.

RESULTS

The results of the experiment are shown in Figure 1. The control sera showed some daily fluctuations in enzyme levels. The SGO-T of the control animals averaged 36.35 units/ml. and the SGP-T averaged 40.8 units/ml. In Figure 1 the average of each transaminase level for that bleeding of the noninfected animals was set at 100% to eliminate the effect of the fluctuation. The enzyme concentration of the serum of the infected rabbits was then calculated as percentage of the controls for each bleeding.

The SGO-T concentration in the infected animals began to rise on the first day and reached its highest point in 3.5 to 5 days. A definite drop in the level of SGO-T was noted at 6 to 7 days post-infection. This drop was followed by a sharp rise just prior to the death of the host at 8.5 to 9.5 days post-infection.

The SGP-T level in the infected animals also rose gradually, reaching a first peak in 4 to 5 days. The SGP-T concentration remained elevated and also showed a distinct rise prior to the death of the host.

DISCUSSION

Following a lethal infection with **Ascaris suum** in an unnatural host (rabbit), there was a definite increase in the SGO-T and SGP-T levels. Andrews et al (1) found that following a sub-lethal infection in swine, the SGO-T level showed a double peak in enzyme concentration, and the SGP-T level showed a single peak in enzyme concentration. Upon recovery from the infection, the SGO-T and the SGP-T levels in the swine returned to normal.

In the rabbits both enzymes showed two distinct peaks, with the second peak coming just prior to the death of the animal. In comparison to the SGO-T levels in the swine, the SGO-T levels in the rabbits showed a shorter interval between the two peaks in enzyme concentration. In the SGP-T levels in the rabbits the first peak is similar to that found in the swine. However, in the rabbits there was an additional rise in the SGP-T concentration sub-terminally.

SUMMARY

The effect of the migration of a lethal number of larval swine ascarids **(Ascaris suum)** on the serum glutamic-oxaloacetic transaminase (SGO-T), and serum glutamic-pyruvic transaminase (SGP-T) levels in an unnatural host (rabbit) was studied.

The SGO-T concentration in the infected rabbits began to rise on the first day and reached its highest point in 3.5 to 5 days. The SGP-T level also rose gradually, reaching a first peak in 4 to 5 days.

The SGP-T concentration remained elevated and showed a second distinct rise just prior to the death of the host at 8.5 to 9.5 days post-infection.

Published with approval of the director, North Dakota Agricultural Experimental Station.

BIBLIOGRAPHY

1. Andrews, M. F., McIlwain, P.K., Eveleth, D. F., Serum Transaminase and Aldolase During Migration of Larval **Ascaris suum** in Swine. Am. J. Vet. Res., 22, 1961, p. 1026-1029.
2. Cabaud, P., Leeper, R., Wroblewski, F., Colorimetric Measurement of Serum Glutamic Oxaloacetic Transaminase. Am. J. Clin. Path., 26, 1956, pp 1101-1105.
3. Karmen, A., Wroblewski, F., LaDue, J. S., Transaminase Activity in Human Blood. J. Clin. Invest., 34, 1955, p. 126.
4. Schloss, M. F., A Resume on Transaminase. Am. J. Med. Technol., 25, 1959, pp. 47-52.
5. Wroblewski, F., and Cabaud, P., Colorimetric Measurement of Serum Glutamic Pyruvic Transaminase. Am. J. Clin. Path., 27, 1957 pp. 235-239.
6. Wroblewski, F., and LaDue, J. S., Serum Glutamic Pyruvic Transaminase in Cardiac and Hepatic Disease. Proc. Soc. Exper. Biol. and Med., 91, 1956, pp. 569-571.

THE DISTRIBUTION AND SIGNIFICANCE OF TWO ENZYMES STUDIED IN THE SPLEENS OF NORMAL RATS[1]

James C. Pettersen
Department of Anatomy
University of North Dakota, Grand Forks, North Dakota

ABSTRACT

The distribution of non-specific esterases and acid phosphatases was studied in the spleens of twenty untreated, male adult rats.

Both enzymes were found in large quantities in the macrophages of the red pulp cords and in scattered, isolated macrophages within the white pulp nodules.

Acid phosphatase was found in cells at the level of the marginal sinus, whereas esterase activity here was slight. Lining cells of the red pulp sinuses showed a constant esterase activity but no activity for acid phosphatase.

It is believed that these two enzymes are characteristic for active macrophages and that potentially active macrophages begin synthesizing them upon stimulation.

[1]The materials used in this work were purchased with grant No. 407 from the American Medical Association. Some of the work was supported by a National Science Foundation Summer Fellowship for Graduate Teaching Assistants.

PHOSPHORYLASE ACTIVITY IN PERIOSTEUM AND MUSCLE OF NORMAL AND LATHYRITIC RATS[1]

Vernon L. Yeager and Malva F. Bach

University of North Dakota, Grand Forks, North Dakota

Department of Anatomy

ABSTRACT

Sixteen weanling and young adult rats were given 150 mg of beta-aminopropionitrile (BAPN) per 100 ml drinking water. After one, two, four, seven, and eight days, they, along with controls of similar age and sex, were killed and the adductor longus and pectineus muscles with their periosteal insertions were removed, frozen with dry ice, cut at 10 micra in a Pearse-Slee cryostat, and stained for amylo-phosphorylase using the technique of Takeuchi and Kuriaki.

The cells of the periosteum from all animals were negative for phosphorylase. All fibers of the pectineus exhibited uniform, intense staining, while different fibers of the adductor longus reacted intensely, moderately, lightly, or not at all. No difference in staining was observed between lathyritic and normal animals or between weanling and young adult animals. BAPN did not inhibit phosphorylase activity in normal tissues **in vitro.**

These results indicate: (1) that periosteal cells either do not store glycogen or that a different pathway of glycogen synthesis is being utilized, and (2) that some muscle fibers do utilize the phosphorylase-linked glycolytic pathway and some, apparently, do not.

[1]This work was supported by research grant No. A-4748 from the National Institute of Health, United States Public Health Service.

CHRONIC OCCLUSION OF THE VENA CAVA ABOVE THE RENALS IN THE DOG

Allan C. Hoekzema, Robert E. Hanson and B. DeBoer

Department of Pharmacology

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Complete inferior vena cava ligation above the renals in dogs has previously been accomplished in short term occlusion experiments and chronically, in multistaged experiments. A number of articles have appeared in the literature which assert that sudden obstruction of the inferior vena cava above both kidneys is nearly always fatal in dogs. It has been our experience that a one stage, complete ligation at this site is viable in nearly all of the dogs in

which the procedure has been attempted. Twenty-one of the twenty-five animals survived the procedure. Various physiological effects of the occlusion were studied. Marked increases of femoral venous pressure with intense venous congestion in the kidneys was observed immediately after ligation. Venous pressure falls as the existing collateral veins distend and allow the blood to return to the heart. When the rate of venous enlargement is sufficiently rapid the congestion in the kidneys diminishes and urine formation is re-established. Death is apt to occur from uremia however if insufficient collateral circulation results in prolonged kidney congestion. Autopsies were performed on all of the animals and the developed collateral pathways studies. Kidney tissue was submitted to the pathology department for examination.

"TRIGGER" MECHANISM IN ENDOTOXIN SHOCK

James A. Vick[1]

Although it is well established that endotoxin induces peripheral vasoconstriction and vasodilation, the "trigger" mechanism responsible for these changes is poorly understood. The present study suggests that the initial vascular contraction is due to an interaction between endotoxin and a component or components of blood. A reproducible bioassay demonstrating this interaction was performed by placing freshly isolated, canine, saphenous veins in an oxygenated saline bath at 37° C., permitting rapid changes of perfusion fluids. A modified Sanborn strain gauge recorded changes in vessel tension. **Escherichia coli** endotoxin was added to the bath in a concentration of 10-20 μg./ml. Blood and its components were obtained from donor dogs.

Results: 1. **Perfusate of whole blood.** Within 30 to 60 seconds after the addition of endotoxin, 5 to 10 gm. of tension was developed by the contracting vessel, which was sustained for 5 to 8 minutes. 2. **Perfusates of Ringer's, Krebs'-Henseleit's, and dextran solutions.** No contraction resulted. 3. **Role of platelets.** A "platelet-rich" serum or plasma resulted in good contraction, but none occurred with a "platelet-poor" serum or plasma. 4. **Heat-labile plasma component.** Contraction did not occur when a buffy coat, rich in platelets, was added to Krebs'-Henseleit's solution. A heat-labile factor in serum or plasma was essential for contraction. Activity was retained when plasma was filtered through sintered glass, but it was lost when passed through a Seitz filter. Dialysis of platelet-rich plasma against Krebs'-Henselheit's solution resulted in an active dialysate as well as a retained reactivity in the ultrafiltrate. 5. **Miscellaneous observations.** A perfusate of defibrinated blood resulted in contraction, but none occurred with canine cerebrospinal fluid.

[1]Surgical Research Laboratory, Ancker Hospital, St. Paul, Minn.

The vessel contraction occurring under the described experimental conditions was believed caused by enzymatic activity, and involved an interaction between endotoxin, serum or plasma, and platelets, which resulted in the liberation of histamine or a histamine-like substance. It is emphasized that in that intact animal, humoral substances, other than histamine, are also involved in the later stages of endotoxin shock and include serotonin, epinephrine, norepinephrine, and acetylcholine.

ULTRAVIOLET SPECTROPHOTOMETRIC STUDIES ON THE Ka OF ACETOACETALDEHYDE

Donald K. Brandvold and F. H. Rathmann

North Dakota State University of Agriculture and Applied Science
Fargo, North Dakota

Acetoacetaldehyde is the first member of the beta-ketoaldehyde series. This chemical is attractive as a possible intermediate in the combustion of hydrocarbons, as a transient in metabolic processes, and as a precursor for organic syntheses. Since acetoacetaldehyde in the pure form is unstable at room temperature, the sodium derivative was employed in making these studies. The objective was to study the ketoenol tautomerism and to measure the enol-acid dissociation of acetoacetaldehyde. Two methods were used: the first used measurement of pH during titration of solutions of the sodium salt; the second method depended on spectrophotometric measurement of the absorption peak at 2,800 angstroms when dilute solutions of the sodium salt were treated with acids and bases.

Sodium acetoacetaldehyde was prepared in absolute ether by reacting alcohol-free sodium ethoxide in equimolar quantities with acetone and methyl formate. This salt was stable at room temperature, but very deliquescent, and no practical method of recrystallization has yet been developed. Previous studies indicate that acetoacetaldehyde exists as a keto-enol tautomer and may occur in four forms at equilibrium: the keto-aldehyde, the aldo-enol, the keto-enol, and the di-enol, of which the keto-enol is reported as most stable. In the enol form, acetoacetaldehyde ionizes, and ionization is assumed to be instantaneous with the formation of the enol structure.

In the first method used for study of keto-enol tautomerism, titrations of solutions of the sodium salt were made, and pH measured as titration progressed. From the data, the pK can be calculated according to

$$pK = pH - \log \frac{\mathbf{C}\ \text{salt}}{\mathbf{C}\ \text{acid}}$$

By this method, value for reference pK of 6.79 was obtained

previously. This procedure is subject to interference caused by acetoacetaldehyde going to triacetyl benzene, which is a second order reaction that can be neglected in dilute solutions which is the basis for the following.

Earlier experiments indicated that solutions of sodium acetoacetaldehyde showed a strong absorption in the ultraviolet spectrum at 2,800 A (0.83 absorbance). This absorption was assumed to be due to the dissociated enol form. The addition of excess acid reduced this peak to a minimum absorbance of .12. The addition of base to the sodium salt solution increased the peak slightly. By making use of these properties, the pK could be studied.

The solutions of the sodium salt were .001 normal. The purity of the salt had previously been determined by fusion with sulfuric acid and measurement of sodium as sodium sulfate. As shown in the following Table 1, it was found that the height of the absorption curve was inversely proportional to the amount of acid added. By measuring both the absorption and the pH of a solution, it was possible to calculate the pK, using the equation that has been given. The concentration of the enolate is equal to the concentration of the sodium salt, and that of the enol to the amount of acid added. By assuming that at maximum absorption all acetoacetaldehyde is in the dissociated enol form, the pK can be calculated. The average value for the pK obtained was 6.85, corresponding to a Ka of 1.4×10^{-7}.

TABLE 1
Data from treatment of .0001 solutions of sodium acetoacetaldehyde

ml. .01 N HCl	pH of solution	Absorbance	pH calc.	Ka. calc.
—	7.25	.76	—	—
.0568	7.15	.71	6.95	1.1×10^{-7}
.1136	7.0	.69	6.87	1.38
.1704	6.8	.58	6.85	1.40
.2272	6.5	.45	6.83	1.47
.2840	6.0	.39	6.83	1.47
.3408	5.3	.20	6.80	1.58
.3976	5.0	.12	6.79	1.60

ml. .01 N NaOH	pH	Absorption
.0568	7.4	.81
.1136	7.6	.83

BIBLIOGRAPHY

1. Dahm, D., Roger Johnson, and F. H. Rathmann. Proc. N. Dak. Acad. Sci., v XII, 1958, pp. 19-23.
2. Dahm, D. and F. H. Rathmann. Proc. N. Dak. Acad. Sci, v. XIII, 1959, pp. 37-41.
3. Rathmann, F. H. and Irene Swanson. Unpublished work.

DETERMINING AIR CLEANER EFFICIENCY BY USE OF RADIOACTIVE ISOTOPES

Ernest L. Multhaup

Department of Mechanical Engineering

University of North Dakota, Grand Forks, North Dakota

The air about us is not pure, but is filled with millions of tiny dust particles which come from many sources: from the ground, products of combustion, rubber particles from tires, and sawdust—to mention a few. It is a common occurrence to see a beam of light literally filled with dancing specks of dust. The specks that can be seen actually make up a small percentage of the total number present because the greater part of atmospheric dust is made up of particles less than five microns in diameter. Particles less than 10 microns in diameter are invisible to the naked eye (1). Smoke is visible as a cloud; yet individual particles are not visible as they range from .001 to .4 microns in diameter (2).

In order to gain some appreciation of the effect of dust particles present in the air, it can be pointed out that a dust loading of 2,000 particles per cubic centimeter of air will obscure a mountain 50 miles away while 100,000 particles per cubic centimeter will limit visibility to one mile. There are thousands of dust particles in every cubic centimeter of air in most places in the world (3). This dust can be called aerosol.

The presence of dust in the air has given rise to an air cleaner industry, and some manufacturers concentrate solely on the production of machines and processes that will remove aerosol from the air. Currently, the person buying an air cleaner does not have a universal index that he may use in comparing different types of air cleaners or cleaners from various manufacturers. The manufacturer cannot point to the efficiency of his cleaner and that of his competitor's, and claim that his is better. Each manufacturer has his own methods of testing and rating his particular type of cleaner, and each test varies in some way from the others.

Disagreement occurs chiefly because of dust particle size, dust particle color, dust particle shape, weight per dust particle, and how well test rating indicates the cleaner's performance. Even if the relative importance of all factors were agreed upon, it still becomes a formidable task to derive a procedure for evaluating these factors, and then obtaining general acceptance.

In the early 1920's air cleaner efficiencies were determined. At that time, quantities of air were drawn from above and below the air cleaner and allowed to impinge upon a glass plate coated with a viscous film which was then examined through a microscope and the particles counted. This was a long and tedious procedure, and

the weight and volume of the particles were ignored. Later, a weight method was used where the weights of aerosol in a given amount of air from both above and below the air cleaner were determined.

Since the development of high-efficiency air cleaners, particularly electronic precipitators that are effective in removing very small particles in the range of ¼ micron, the weight method fails to give a true picture of the air cleaner's efficiency. For instance, one 70-micron particle will weigh more than 12 million particles of ¼-micron diameter. It is primarily the very small particles that do not settle out on horizontal surfaces which cause most of the discoloration to walls and fabrics. Small particles exhibit a more random movement, rather than a vertical settling movement, and are more prone to come into contact with wall surfaces. The small particles have more surface area per unit weight and are capable of more discoloration per unit weight than larger particles. Many air cleaner installations are made to prevent discoloration, and the efficiency rating should be indicative of their ability to remove small particles. In order to determine air cleaner efficiency in removing aerosol which causes discoloration, a blackness test has been devised. There are variations of the method, but basically dust is collected from upstream and downstream of the cleaner, and the blackness or discoloration is estimated by measuring the intensity of transmitted light through the collected aerosol. The method has variations from manufacturer to manufacturer, and the equipment needed for accurate and reproducible results is expensive.

The National Bureau of Standards has a standard blackness test which is reported to be satisfactory and gives reproducible results. In our laboratory a manufactured model using this principle of measuring blackness, called the Dill Dust Spot Tester, has not proved satisfactory. Many results are not even reasonable, and reproducibility has not been approached.

EXPERIMENTAL

It was proposed that the aerosol be tagged with a radioactive tracer, and by reading the radiation intensity both before and after the air cleaner, a measurement of the airborne dust be obtained and an estimate of the air cleaner efficiency made. The use of radioactive isotopes in this manner to determine air cleaner efficiency has not been investigated by other experimenters.

Phosphorus-32 was chosen as the radioactive tracer because of its safety, easily counted high-energy beta radiation, and general availability. It is relatively safe since the radiation is easily-shielded beta radiation, and the half life is only 14.3 days. Radioactive waste can be stored for several months, and then disposed of normally when the radiation level has subsided.

Aerosol was prepared by pulverizing anthracite coal to a maxi-

mum particle size of 53 microns, and then forming a mixture of 80 percent coal and 20 percent lampblack. This type of aerosol closely approximates atmospheric dust. The aerosol was made radioactive by mixing with phosophorus-32 in the form of H_3PO_4 in HCl solution diluted to mixing proportions with ethyl alcohol.

Two methods of making the measurements were investigated. In the first, the radiation intensity, in counts-per-minute for the air stream in the duct, both before and after the air cleaner was measured. In the second, aerosol samples were collected on filters papers by withdrawing equal volumes of air both before and after the air cleaner, and passing the volumes through separate filter papers. The airborne dust was deposited on the filter papers, the radiation intensity was then measured and the collection efficiency calculated from the values obtained.

RESULTS

The first method proved to be unsatisfactory because radioactive dust contaminated the duct and gave an ever-increasing and very large background count.

The second method gave very satisfactory results. Dust collection efficiency values were found with a maximum of 99 percent at 200 fpm face velocity to a minimum of 90 percent at 600 fpm face velocity. These values are in the same range as values quoted for electronic precipitators of the type used in the tests. The decrease in collection efficiency as the face velocity increases is reasonable, as some of the heavier particles and some of the particles in the most adverse positions approaching the collector plates would be carried through without opportunity to adhere to a collector surface.

Since discoloration of surfaces is caused by the lighter and smaller particles, which have much surface area per unit weight, it would seem that the method of using radioactive tracers would be ideally suited. It is reasonable to assume that the solution containing the radioactive tracer will cover the surfaces of the dust particles reasonably uniformly. The count rate will then be directly proportional to the amount of surface area, and hence the discoloration potential of the aerosol.

A high degree of reproducibility was found by using the second method. Equal volume air samples are taken and passed through the test filter papers; the filter papers are then counted with a radiation detector, and there is no human value judgment which may vary the results. This is not the case with the Dill Dust Spot Tester which requires that a galvanometer needle be balanced, moving first in one direction and then in the other, with the average of the two readings being recorded. If the needle is moving faster in one direction than in the other, then there will be an error. For a universally acceptable test, the possibility of variation due to operator

technique is not desirable. It is believed that the second radioactive tracer method is suitable for a standard test.

CONCLUSIONS

There are some disadvantages that are recognized in the method of using radioactive tracers for estimation of dust loadings. Radioactive isotopes are fairly expensive, but so are other methods of testing. Radioactive waste disposal in this case is more an inconvenience than a problem, as all that is needed is a metal container in which to store the radioactive waste material while it deteriorates to a safe level. It is possible with some methods of testing to use naturally occurring atmospheric dust to determine air cleaner efficiency, whereas, using radioactive tracers necessitates the use of a prepared aerosol and injecting it into the air stream. Using naturally occurring aerosol, however, takes hours for each test, whereas injecting a prepared aerosol into the air stream makes the test length a matter of minutes.

Investigation of test procedures is being continued, and a standard test will no doubt ultimately be developed. There is a possibility that a form of the radioactive tracer technique will finally be shown to be best.

BIBLIOGRAPHY

1. Jannings, Burgess H., and Samuel R. Lewis, Air Conditioning and Refrigeration, 3d ed., International Textbook Company, Scranton, Pr., p. 425, 1949.
2. Bulletin No. 510, American Air Filter Company, Inc., Louisville 8, Ky., p. 4.
3. Blacklin, S. Cyril, Dust, Sherwood Press, Cleveland, Ohio, p. 71, 1934.

A METHOD FOR THE STUDY OF AMINO ACID TRANSPORT IN THE SMALL INTESTINE[1]

F. A. Jacobs and W. G. Tarnasky

University of North Dakota, Grand Forks, North Dakota

A revision has been made in the procedural technique which extends the scope of investigations on the absorption of amino acids in the small intestine. Previously, the amino acid solution was recirculated through the upper small intestine for an hour. The equipment has been altered to allow the perfusing solution to make only

[1]Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, School of Medicine. Investigations were supported in part by the NIH Research Grant A-2023.

a single pass through the intestine, after which the rate of absorption is measured by determination of residual amino acid in the perfusing solution.

The advantage of the new system is that the experiment can be more closely regulated since the concentration of the amino acid being perfused will remain constant. Also, the time required is reduced, and consistent and reliable results can still be obtained.

Utilizing the new procedure, a rate-study has been carried out on the intestinal absorption of L-tyrosine-14C, both in relation to the influence of varying concentration and varying the time interval of perfusion. In addition to measuring the total transport across the intestinal wall, we have made studies of the intestinal uptake, by dissolving the intestinal segment in 5N KOH and measuring an aliquot for radioactivity employing liquid scintillation techniques.

AN EFFECT OF MICROORGANISMS IN THE STUDY OF INTESTINAL ABSORPTION[1]

F. A. Jacobs, D. Person, R. C. Flaa and R. M. Marwin

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

In our previous investigations on the intestinal absorption of the nutrients L-tyrosine and D-glucose we found what appeared to be a "second system" of active absorption. There was a marked increment in the percentage of these substances disappearing from the perfused upper small intestine of the intact rat in instances in which the perfusate (amino acid or glucose) concentration was less than the level found in the blood. This "second system" involved in the utilization of these nutrients was found to be directly related to the population of microorganisms present in the perfusion system. This influence of microbial action was of little significance when higher levels of the nutrients were studied, but was of real consequence at levels at (or less than) their respective concentrations in the circulation. These investigations were supported in part by NIH Research Grant A-2023.

---

[1]Guy and Bertha Ireland Research Laboratory, Department of Biochemistry and Department of Bacteriology and Immunology, School of Medicine.

ELECTROPHORETIC SEPARATION AND GRAVIMETRIC AND COLORIMERIC ANALYSIS OF THE BLOOD SERUM PROTEIN OF *RANA PIPIENS*[1]

Jay W. Constantine[2] *and Orrle O. Stenroos*

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

Separation of proteins, lipoproteins and glycoproteins in the blood serum of **Rana pipiens** was achieved by paper electrophoresis. Total protein was estimated by gravimetric and colorimetric analysis.

The protein fractions included albumin as the fastest migrating fraction with three other fractions which are thought to be similar to mammalian serum globulins. The mean relative per cent values obtained for the fractions starting with the fastest migrating fraction to the slowest are: 41.6 ± 6.42%, 22.4 ± 1.16%, 16.2 ± 1.14% and 19.6 ± 1.40%, respectively. The mean migration distances are 65±2.60 mm., 43.5 ± 2.66 mm., 12.3 ± 1.54 mm. and −1.8 ± 3.42 mm. The minus sign denotes net cathodal migration.

Usually only one lipoprotein fraction was obtained which migrated toward the anode a mean distance of 11.2 ± 1.43 mm. from the origin. At times two fractions were separated. The leading fraction was larger and migrated a mean distance of 11.0 ± 1.24 mm. while the second fraction migrated 5.5 ± 2.18 mm. The mean relative per cent values for the fastest and slowest migratory fractions are 52.7 ± 14.60% and 47.3 ± 14.60%.

The paper electrophoretic strips stained for gycoproteins had four fractions for which the means of the migration distances are, 70.0 ± .80 mm., 45.7 ± 1.32 mm., 13.9 ± .74 mm. and 3.9 ± .74 mm. The mean relative per cent values starting with the fastest migrating to the slowest are 16.0 ± 2.16%, 54.6 ± 3.86%, 15.7 ± 3.86% and 13.9 ± 2.42%, respectively.

With the colorimetric analysis the mean total protein value was 2.65 ± .04 gm per 100 ml serum compared to 2.76 ± .70 gm per 100 ml of serum for the gravimetric procedure.

The serum proteins of **Rana pipiens** are similar to mammalian serum proteins in that there is present albumin and the globulins. The lipoprotein paper strips do not show any lipoproteins in the region which corresponds to the α lipoproteins in mammalian serum. However, the glycoprotein pattern is much like that for human serum. The total protein content in **Rana pipiens** is approximately ⅓ of that in human serum.

[1]This work supported in part by research grant (248) North Dakota Heart Association.

[2]Present address: Charles Pfizer and Company, Groton, Connecticut.

REACTION OF SULFUR YLIDS WITH NITROSOBENZENE

A. William Johnson

Department of Chemistry

University of North Dakota, Grand Forks, North Dakota

Our original work showed that dimethylsulfoniumfluorenylide (I) reacted with carbonyl compounds to form epoxides (oxiranes). It was expected that I would also react with nitrosobenzene and, on mechanistic grounds, would form oxaziranes. Instead, nitrones have been isolated from this reaction in near-quantitative yields.

Since some of the by-products formed in the reaction of I with carbonyls were accounted for by the decomposition of the ylid to a carbene, the reaction of the resulting carbene with nitrosobenzene might have afforded the nitrone. Alternatively, the oxazirane might have been the initial product only to have been isomerized to the nitrone under the reaction conditions.

Carbenes were found to react with nitrosobenzene but afford only nitrones. Oxazirane, if formed from reaction of the ylid or carbene with nitrosobenzene, must be a very transitory intermediate which spontaneously rearranges to the nitrone.

ON THE GEOMETRY OF A CERTAIN CLASS OF WIRE PUZZLES

Charles Hatfield

University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Wire puzzles of the class considered can be described as consisting of at least three pieces interlocked in such a way as to seem essentially 'connected.' One of the pieces, however, can be extricated from the rest of the linkage; this is the puzzle: separate that piece.

These puzzles apparently have a common ancestor, as shown in figure 1.

Here the piece labeled 'C' is separable from the rest of the puzzle. A and B are necessarily linked together and cannot be separated without violence. The fundamental geometrical property involved in these puzzle-types is usually met for the first time in advanced calculus, but can be easily explained without that background. We say that a region R of the plane is 'simply connected' if one can shrink continuously a curve C in such a way that no points not

in R are encountered. If this is not possible, the region is said to be 'multiply connected'; that is, there is in a multiply-connected region R at least one curve C such that shrinking it to a point will

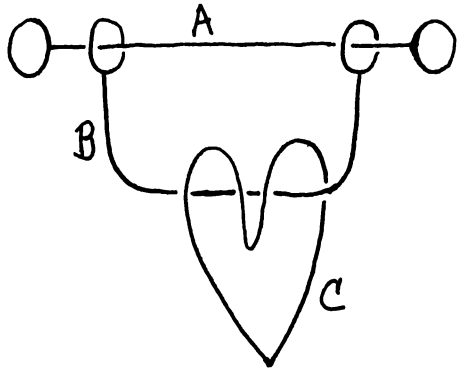


Fig. 1

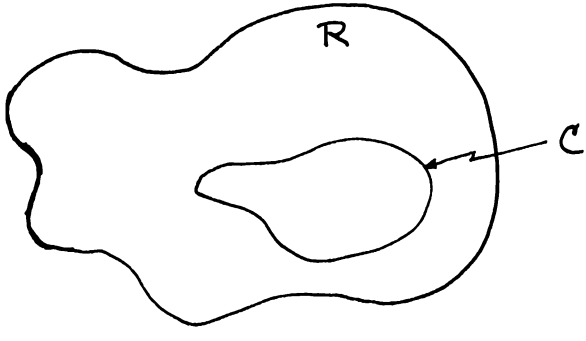


Fig. 2

take one through points not in the region R. Such a region is aptly described as doughnut-like and is called a torus. The curve C, it should be noted, is always a 'simple closed curve'; that is, one which, despite its twists and turns; can be made from a circle of the proper circumference. In figure 2 we have an example of a simply-connected region R, and in figure 3 a multiply-connected region R.

By means of this distinction between regions, wire puzzles can be designed with confidence that there is a solution before it is attemped. Indeed, this is often the real challenge. It is known mathematically to have a solution, and yet one does not know how to

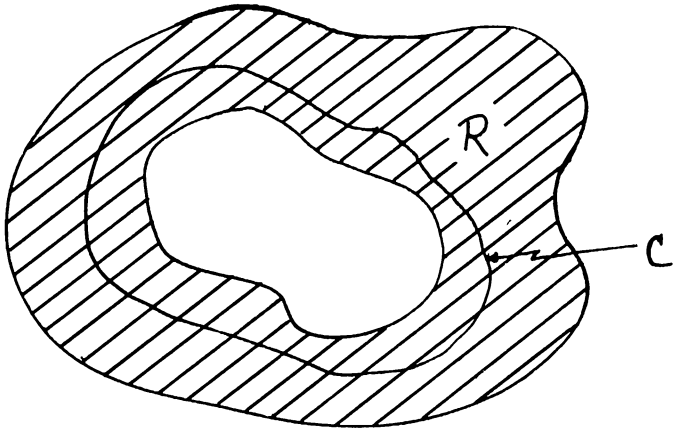


Fig. 3

go about solving it. There is a simple generalization of the puzzle in the first diagram attached: simply increase the number of pieces 'B' as desired. This increases the difficulty of the puzzle, and when the number gets too large (say 3 or 4), one must replace 'C' by a loop of string or some such flexible material. Topologically, we say, the puzzle is still in the same category, no matter how many pieces 'B' are introduced.

One of the puzzles that is topologically equivalent is called "the tiring irons." It may have any number of rings. In figure 4 is one that has five rings. Here the object of the puzzle is to remove the loop 'C' by 'dropping' or 'raising' the various rings in mechanically allowable positions. For example, counting from the right end, no ring can be dropped through loop C (except the first two) unless the only other ring on C before it is its immediate neighbor. A similar rule obtains for the reverse process of restoring the rings to the position in the figure.

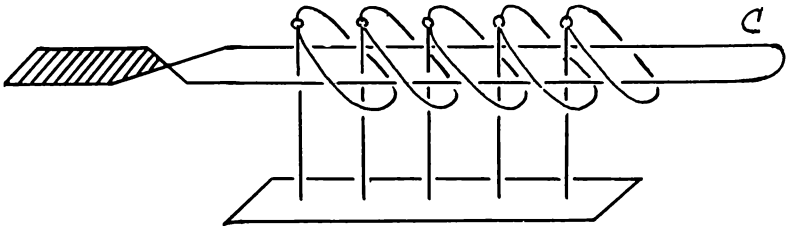


Fig. 4

If the upper position is designated by the number 0 and the lower or dropped position is designated by the number 1, then the working of the puzzle essentially takes one through positions which are represented by certain binary numbers between 0 and 11, 111 or 0 to 31 in base 10.

Some of the binary numbers correspond to ring positions not used. Excluding these unused positions, we arrive at the number of moves R_n to remove the n rings: $R_n = \frac{1}{3}(2^{n+1} - 2)$ for n even, $R_n = \frac{1}{3}(2^{n+1} - 1)$ for n odd; e.g., $R_1 = 10$, $R_5 = 21$.

FLUORESCENCE MICROSCOPY — A NEW DIAGNOSTIC TOOL IN NORTH DAKOTA

A. A. Gustafson and James B. Hundley

Department of Health

University of North Dakota, Grand Forks, North Dakota

The basic research for fluorescent microscopy and the use of fluorescien goes back to 1905 (1), but not until 1930 were successful attempts made to alter antibody protein. This was soon followed in 1933 by the work of Hopkins and Wormall (2) who attached phenol isocyanate to protein molecules and studied their immunological reactions.

The present technique for tagging antibodies so that the antigen-antibody reactions can be observed by fluorescence microscopy was originated by Coons and co-workers (3) at Harvard in 1942. The work of Goldman (4) who employed the FA technique for the differentiation of **Entomoeba histolytica and Entomoeba coli,** suggested the potential applications of FA techniques in the diagnostic field. Subsequently, fluorescent antibody studies in the field of diagnostic bacteriology were initiated, and the first publications by Moody and Thomason (5) appeared in 1956.

Equipment

A Leitz Labolux fluorescent microscope is equipped with a high pressure mercury arc lamp enclosed in a quartz envelope, together with filtering systems necessary for the three general lighting systems:

(a) Near ultraviolet exciting light between 350-400 Mμ; secondary filter colorless but opaque to ultraviolet.

(b) Blue violet exciting light between 400-450 Mμ; secondary filter distinctly yellow.

(c) Combined ultraviolet and blue violet exciting light between 350-450 Mμ; secondary filter distinctly yellow.

It is possible to use either a bright-field or dark-field condenser, but generally FA is much easier to use and much more effective with a dark-field condenser. Ordinary achromatic lenses are suitable but apochromatic or fluorite lenses give a brighter image. The use of a binocular head may result in the loss of about 50 percent or more of image brightness as afforded by a monocular head.

No attempt has been made to prepare our own fluorescien tagged antisera. We have relied on antisera supplied by the Communicable Disease Center or obtained commercially from Difco Laboratories, Baltimore Biological Laboratories, or Sylvana Company. In all instances, commercial reagents have been checked against control lots from the Communicable Disease Center and have been found to be satisfactory.

Experimental

Several techniques have been described for using fluorescien tagged antibodies. The basic procedure is the direct method used in our routine for streptococci. The direct method (6) consists of bringing fluorescien tagged antibodies in contact with antigens fixed on a slide, allowing them to react, washing off the excess antibody, and examining the slide by dark-field illumination using an UV light source. The labeled antibodies will be absorbed onto their homologous antigens and take the form of the antigen particles appearing as fluorescent bodies. In the case of streptococci they will appear as brightly fluorescent sphericle bodies arranged in chains. For streptococci, the combined ultraviolet and blue violet exciting light between 350-450 Mμ with a secondary filter distinctly yellow is used.

The fluorescent antibody technique was established as a routine procedure in the Grand Forks Public Health Laboratory on July 17, 1961. All throat swabs received are incubated in Trypticase Soy Broth for two hours. At the end of this time the cotton tip swab is placed in 2 ml aliquots of sterile physiologic saline. The swab is agitated vigorously to wash any bacteria from the cotton. Excess saline is removed by forcefully ringing the swab on the edge of the tube; the swab is then discarded. The saline in the tube is centrifuged at 2,000 RPM for 5 minutes. The supernatant is discarded and the sediment smeared onto a glass slide. The slide is allowed to dry and is then treated with **Group A Streptococcus** antisera in the manner described for the Direct FA test.

In rabies diagnosis the use of FA has been limited to those animal heads submitted directly to the Public Health Laboratory for examination. Brain material submitted or stored in a glycerine solution is unsatisfactory for this procedure. We have set up a new rabies collection system which provides that all human exposure specimens will come directly to the Public Health Laboratory, allowing us to do fluorescent antibody studies on all human exposure rabies specimens.

As of December 1, 1961, the FA technique has also been applied to stool specimens for the detection of **Enteropathogenic Escherichia coli** in infants under 2 years of age. This group of organisms is responsible for large numbers of cases of infant diarrheas, both in epidemics and in single cases.

RESULTS

During the period of July 1, 1961, through March 28, 1962, a total of 1,649 throat cultures were examined by FA; of this number 292 were positive for **Group A Beta hemolytic streptococci,** an isolation rate of 17.7 percent. Table 1 shows the number and percentage of isolations by month during this period. It will be noted that a considerable increase in frequency of isolation occurs during January, February, and March, which are usually the months of highest incidence of streptococcal disease.

TABLE 1
Group A Streptococci Isolated by FA
July 1, 1961, to March 28, 1962

	Throat Cultures	FA Positive	Percent Positive
July 1961	49	5	10.2
August 1961	101	7	6.9
September 1961	122	12	9.8
October 1961	211	13	6.1
November 1961	243	29	11.5
December 1961	179	21	11.5
January 1962	228	53	23.2
February 1962	297	63	21.2
March 1962	219	89	40.6
Total	1,649	292	17.7

Comparison of these throat culture results with previous experience using conventional bacteriology is startling. During the fiscal year of July 1, 1960, through June 30, 1961, a total of 210 **Beta hemolytic streptococci** were isolated from 2,402 throat specimens or an isolation rate of 8.7 percent.

At the present rate of isolation with FA of 17.7 percent, over twice as many **Beta hemolytic streptococci** are isolated. It is probable that of the **210 Beta hemolytic streptococci** isolated, a portion would have belonged to groups other than Group A.

In addition to doubling the isolation rate, the time necessary for returning a positive report to the physician has been materially reduced. With FA, a positive report will be given to the physician on the same day a specimen arrives. With conventional bacteriology as practiced before the advent of FA, at least 48 hours would have elapsed before a report could be given to the physician. As you can see, this gives the physician a report at least 40 hours sooner when the FA is used.

During the period of October 1, 1961, through March 28, 1962, 34 animal heads have been examined for rabies by the Fluorescent technique; of these, 4 have been positive—or 11.7 percent.

During the period of November 24, 1961, to March 28, 1962, 77 stool specimens or rectal swabs on infants under 2 years of age have been examined for **Enteropathogenic E. coli.** A total of 12 positives have been found, which includes 7 of the known 10 types or an isolation rate of 15.48 percent.

CONCLUSIONS

1. The use of fluorescent microscopy has resulted in the doubling of streptococcal isolation rate.
2. The time necessary for isolation tests has been materially shortened.
3. The new technique affords a considerable savings in media and work time.
4. In both Rabies and **Enteropathogenic E. coli** cases, insufficient specimens have been examined for comparisons with previous experience.
5. That the use of FA is a sound practical diagnostic tool for routine use in a Public Health Laboratory.

REFERENCES

1. Bogert, M. T. and R. G. Wright, J. Am .Ch. Soc. 27, 1310-1316.
2. Hopkins, S. J. and A. Wormal, Biochem. J. 27, 740.
3. Coons, A. H., H. J. Creech, R. N. Jones, and E. J. Berliner, Immunol. 45, 159-170.
4. Goldman, M., Am. J. Hyg. 58, 319-328.
5. Moody, M.D., M. Goldman, and B. M. Thomason, J. Bact. 72, 357, 361.
6. FTA—Fluorescent Tagged Antibody Reagents—Pamphlet 173, Nov. 1961—Difco.

INDOLE-3-ACETIC ACID OXIDASE AND INHIBITOR IN LEAFY SPURGE (*Euphorbia esula L.*)[1]

J. G. Dosalnd, E. A. Helgeson and C. R. Swanson

North Dakota State University of Agriculture and Applied Science

Fargo, North Dakota

ABSTRACT

Aqueous extracts of leafy spurge tissue strongly inhibit the indole-3-acetic acid (IAA) oxidase system of both the Alaskan pea

[1]The work was supported, in part, by the North Dakota Agricultural Experiment Station, Project H-9-1.

(**Pisum sativum L.**) and leafy spurge (**Euphorbia esula L**). When dialyzate of the aqueous extract was added to a reactant mixture, an inhibition of the IAA oxidase occurred. Preparation of an active IAA oxidase from leafy spurge required purification of the crude homogenate.

Exposure of the extract to temperatures of 100°C for several minutes did not destroy the inhibitory action. Inhibitory action of the extracts was removed by a strong anion exchange column, while strong cation exchange resin had no affect upon the inhibitory action. It may be concluded that a thermostable, relatively low molecular weight anion was responsible for the inhibitory action.

The inhibition was typically a lag period in oxygen uptake. At the end of the lag period, rapid uptake of oxygen occurred. The rates of oxygen uptake, after the end of the lag period, were virtually the same as the initial rates of an uninhibited reaction. Manganous chloride and 2, 4-dichlorophenol were cofactors in the reaction. Presence or absence of exogenous substrate had no affect upon the length of lag.

A linear relationship between inhibitor concentration in the reactant mixture and the length of the lag period was not observed. The highest concentrations of inhibitor resulted in complete inhibition of the reaction.

A similiarity between the IAA oxidase system in the pea and leafy spurge was noted. Enzymatic oxidation of IAA by homogenates of leafy spurge occurred only in the presence of added manganous ion and 2, 4-dichlorophenol. The reaction involved an uptake of molecular oxygen and evolution of carbon dioxide. The cofactor requirement suggested that the mechanism and components of the IAA oxidase system in leafy spurge are similar to the IAA oxidase system identified in peas and several other higher plants.