

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF RESEARCH ADMINISTRATION
RESEARCH PROJECT INITIATION

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Date: July 19, 1974

Project Title: **Glucose Utilization of Various Tissues in the Uremic State**

Project No: **E-19-629**

Principal Investigator **Dr. J. J. Smrekar**

Sponsor: **Public Health Service**

Agreement Period: From 7/10/74 Until 5/31/75

Type Agreement: **Biomedical Sciences Support Grant (Internal)**

Amount: **\$5,000**

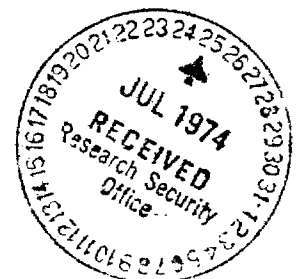
Reports Required: **Final due 7/15/75**

Sponsor Contact Person (s): **Dr. John W. Cranshaw
Biomedical Sciences Support Grant Committee
School of Biology
Campus**

Assigned to: Chemical Engineering

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GEORGIA INSTITUTE OF TECHNOLOGY
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RESEARCH PROJECT TERMINATION

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Date: 17 September 1975

Project Title Glucose Utilization of Various Tissues in the Ureic State

Project No: E-19-629

Principal Investigator: Dr. J. J. Sazekar

Sponsor: PHS, Internal Biomedical Sciences Support Grant under G-32-608

Effective Termination Date: 5/31/75

Clearance of Accounting Charges: 5/31/75

Grant/Contract Closeout Actions Remaining:
None

Assigned to School of ~~Chemical Engineering~~

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E 19-629

Final Report On

GLUCOSE UTILIZATION OF VARIOUS TISSUES IN THE UREMIC PATIENT

Project File Number G-32-608

Project Number E-19-629

Submitted To

John W. Crenshaw, Chairman
Biomedical Sciences Support Grant Committee

Submitted on

June 24, 1975

Prepared by:

Joseph J. Smrekar
Principle Investigator

Abstract

All new supplies and equipment requested under the continuation proposal were purchased early in the year. The equipment was selected because it could meet the immediate need for this specific project and also because it was flexible enough to meet the expected expansions of the project. With the purchase of the new blood chemistry system the procedures were greatly shortened and the results were much more reliable and reproducible.

The procedures were evaluated and it can be reported that a set and workable procedures for the surgical portion and the actual metabolic test run of the original proposal are fixed. The only snag in the total procedure was uncovered recently. The problem involves the liquid scintillation counting of the various tissues, especially the tissues with a high blood content. The problem involves a color quenching which could not be eliminated with a hydrogen peroxide bleechings. The hydrogen peroxide also introduces a chemical quench. Investigations on dry counting of the samples has started and these studies will continue through the summer. Preliminary tests have shown great promise.

Once the counting problem is resolved, the full procedure will be tested and evaluated. If the evaluation is good, a proposal will be submitted to a funding agency in late summer or early fall.

Introduction

The purpose of the renewal grant was to allow for more tests to be conducted using the equipment that was built and to test and evaluate the existing procedures and proposed modifications to the procedures. The proposed modifications were to separate the blood and use only the plasma in the liquid scintillation counting, to wash the tissues with cold isotonic saline to rinse off excess blood to compare the hemoglobin content of the tissue to that of blood to get a measure of the blood that was being counted with the tissue, to look at the possibility of running uric acid determinations as a possible measure of protein metabolism, and finally to look at paper thin layer chromatography as a method of separating the lipid fractions of the blood. The other object of the renewal proposal was to purchase a blood chemistry system that would be reliable, accurate and simple to use and thus fix the blood chemistry procedures. A secondary purpose of the renewal was to test several rats to see if previous trends could be matched.

Results

In regards to the existing procedures, the following can be reported: the surgical and metabolic methods are sound and good. The techniques of the surgery have been adequate by perfected so as not to have an abnormal sample of surgically traumatized subjects. The actual experimental runs including all preliminary preparation and all post sacrifice sample collection are good and can be performed in a routine sequence of steps. Therefore, as far as existing procedures all evaluations were favorable and the procedures are sound. The only setbacks encountered were with some of the modifications.

Before describing the modifications that did not meet expectations, a description of those that did will be presented. First, the cold saline rinse turned out quite favorable. Excess blood was removed and the tissue appeared to be unaltered. Going, hand in hand, with the saline rinse was the comparison of the hemoglobin contents of the rinsed tissue and blood. This modification is also favorable and will be kept as part of the working procedure.

The greatest setbacks were in the analysis area. First, the liquid scintillation unit that was to be used for tissue counting was inoperative for the first two thirds of the year and therefore no evaluation of the plasma counting or rinsed tissue counting could be made until early this calendar year. Unfortunately this was one of the problem areas last year and it proved to be so this year as well. The major problem was color quenching. Because of the blood in the tissues or because of reactions of the tissues with the solubilizer the counting samples were not clear but had various reddish hues to them. Bleaching with hydrogen peroxide was tried but with limited success. The peroxide failed to remove the color or so much had to be added that a chemical quench was obtained. Currently, dry counting is being investigated. The procedure is similar to that used to count the BaCO_3 . A slurry is made of the tissue and solubilizer. This slurry is placed in a counting planchet and the liquid phase is evaporated. This procedure forms a uniform layer of countable material in the planchet which can be dry counted. The advantages of dry counting are that it is a highly efficient technique and that quenching of any type is not a problem. Studies on this dry counting technique will continue through the summer.

The uric acid determination was dismissed as an index of protein metabolism. No conclusive results could be obtained. Creatinine will be looked at as a possible replacement for the uric acid. The use of thin layer

chromatography for lipid identification was dismissed for the time being. It was felt that the techniques involved were too complex and sophisticated. Currently, spectroscopic techniques are being investigated as a possible replacement. If no suitable replacement is found, the lipid separation and identification would be sent out to be done.

Of the subjects that were tested during the year, the same trends existed. A marked glucose intolerance is exhibited along with decreased uptake of the tissues and in the CO_2 expired.

Summary

Overall, the year must be assessed as a good one. Techniques were perfected and a workable and reliable experiment excluding analysis was developed. In regard to the analysis, the dry counting shows much promise. If the dry counting proves to be successful, a proposal to do an in depth study will be submitted to a funding agency.