FINAL TECHNICAL REPORT

Title of Grant: "Investigations of the Inhibitory Effects of Tocopherol (Vitamin E) on Free Radical Deterioration of Cellular Membranes"

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A TECHNICAL REPORT

Studies toward the inhibitory effects of d,l-alpha-tocopheryl acetate on the Free Radical Deterioration of Cellular Membranes.

ABSTRACT

Objectives:

The objectives of this research endeavor are: (1) to establish the inhibitory effects of d,l-alpha-tocopherol and d,l-alpha-tocopheryl acetate on the free radical deterioration of cellular membranes; and (2) the possible level of toxicity of d,l-alpha-tocopherol and d, l-alpha-tocopheryl acetate in mice.

Experimental:

Sixty-four mice were divided into five groups with approximately sixteen mice in each group. Each group was again divided into sub-groups; two cages in each group contained four three month old mice and the third cage in each group contained approximately eight mice which were five months old. All the mice lived in 11.5" X 5" X 7.25" plastic cages which were cleaned daily.

The groups were again divided as follows:

Group A - received 2 grams of vitamin E (d,l-alpha-tocopheryl acetate) per 1 gram of 170290 vitamin E deficient test diet powder.

Group B - received .8 grams of vitamin E (d,l-alpha-tocopheryl acetate) per 1 gram of 170290 vitamin E dificient test diet powder.

Group C - received .8 grams of vitamin E (d,l-alpha-tocopherol) per 1 gram of 170290 vitamin E deficient test diet.

Group D - received 170290 vitamin E deficient test diet powder.

Control Group - received commercial Purina laboratory chow pellets.

The mice ingested the vitamin E orally. The individual weights of the mice were determined by averaging the weights of the entire group and the required amount of vitamin E, agar and water were added to powdered ARS/Sprague-Dowley 170290 vitamin E deficient test diet. Noticeable differences in weights were compensated for by increasing or decreasing the amount of vitamin E added to the deficient test diet.

The necessary precautions were taken to insure that the various preparations remained pure.

The methodologies employed to measure physiological parameters were:

1. Heart rate and blood pressure recordings via a DMP-48 physiograph.
2. Blood clotting and hematocrits.

Discussion

Kutsky[3] reported that large quantities of vitamin E possibly results in an increase in blood pressure; and work in our laboratory supports Kutsky's statement. It was observed that mice in Groups A and B showed systolic physiograph recordings that indicated high blood pressure (Figure I). Figure I indicated blood pressure readings for mice selected at random to be approximately 200 mm Hg. However, the other groups including the control group have readings considerably less than 200 mm Hg (Figure I). When blood pressure was compared to blood clotting time (Figure II) it was noted that mice with high blood pressure showed low clotting time; likewise mice with high clotting time showed low blood pressure readings.

At present, there is not sufficient information to report on hematocrits due to the unavailability of the necessary equipment for this measurement.

Current literature indicates that mice on a vitamin E deficient diet have difficulty reproducing.[4] Group D reproduced but the offsprings were premature and never lived any longer than 24 hours. The offsprings had a very peculiar color which was especially noticeable in their hind legs (observations made in three different litters). There has also been instances where mice in Group D appeared to be pregnant (visual observations), but never completed their pregnancies. It is believed that the fetuses were possibly re-absorbed into the blood stream by some mechanism which was not pursued.

The offsprings of Group A were exceptionally large compared to those of the other groups.

In Figure III, individual food consumption, it was noted that mice in Group A consumed the least (excluding the control group).

Mice in Group A showed considerable weakness and developed partial immobility.

The mice in Group C were small in size but active. Their food consumption was lower than any other group except that of Group A.

During the blood clotting time test, blood is drawn from a vein in the tail by a syringe. At the point where the needle punctures the skin an infection occurs on the tail of the Group D mice. (Illustrations not available.) If the mice in this group were injured, the healing time is slow, even with medication. Apparently, vitamin E deficiency has an effect on test animal's clotting mechanism and there appears to be an inhibition to the healing process.

Studies with vitamin E at other laboratories indicated the lack of vitamin E effects the pituitary glands, reproductive organs, kidneys, muscles and other parts of the body.[2 & 3] Our research team has shown that vitamin E deficiency effects the reproductive system and the life expectancy of the test animals. Work in our laboratory appears to indicate that large quantities of vitamin E results in high blood pressure, muscular problems and eventually termination of life in mice.

However, at present, no conclusive evidence in experimental procedures have been employed to directly indicate that free radical deteriorates fatty tissue. This was observed directly because of the lack of continual support for the project and the unavailability of of an electron spin resonance spectrometer (ESR). However, this is

the subject of future experimentation if funds become available via the present supporting institution in some alternate funding source.

The present research endeavor's are far from being conclusive, and much more study in this virgin area of research must be done. From consultation and re-examination of the scope of the research project it was concluded that the work attempted through very meaningful was much too broad.

Consequently, future endeavors will attempt to narrow the research objectives to one specific problem, i.e., the mechanism of free radical inhibition by vitamin E. This study will utilize ESR techniques for detecting free radicals in fatty tissue.

Future Endeavors

Electron Spin Resonance (ESR). Objectives - Studies on the inhibitory effects of d,l-alpha-tocopherol on the deterioration of fatty tissues by ozone.

The inhibitory effects of tocopherol on free radical deterioration of fatty tissue needs to be narrowed to some specific objective. This narrowing would better contribute to the knowledge of free radical destruction, in an area in which little is known concerning the mechanism of radical scavenging via auto-oxidant.

Therefore, we purpose to study the electron spin resonance spectra of fatty tissue which has been exposed to d,l-alpha-tocopherol and ozone as compared to spectra of fatty tissue and ozone.

Samples of fatty tissues which have been exposed to ozone (in vivo) and d,l-alpha-tocopherol extraced by butonal, will be prepared from sacrificed mice and will be analyzed by ESR spectroscopy.

Ten groups will be studied. Fatty tissue will be extracted from sacrificed mice in the following groups:

Group A - 2 grams of vitamin E (d,l-alpha-tocopheryl acetate) per 1 gram of 170290 vitamin E deficient test diet powder.

Group B - .8 gram of vitamin E (d,l-alpha-tocopheryl acetate) per 1 gram of 170290 vitamin E deficient test diet powder.

Group C - .8 gram of vitamin E (d,l-alpha-tocopherol) per 1 gram of 170290 vitamin E deficient test diet.

Group D - 170290 vitamin E deficient test diet powder.

Control Group - commercial Purina laboratory chow pellets.

ESR Spectra of fatty tissue and the blood of these animals will be investigated to determine any positive or negative occurances in their spectra. From the ESR spectra it is anticipated that a mechanism will be proposed which will possible help to delineate a mechanistic pathway to explain the deterioration of fatty tissue by free radicals and the inhibition of free radicals to the deterioration of fatty tissue by tocopherol.

Figure 1

BLOOD PRESSURE

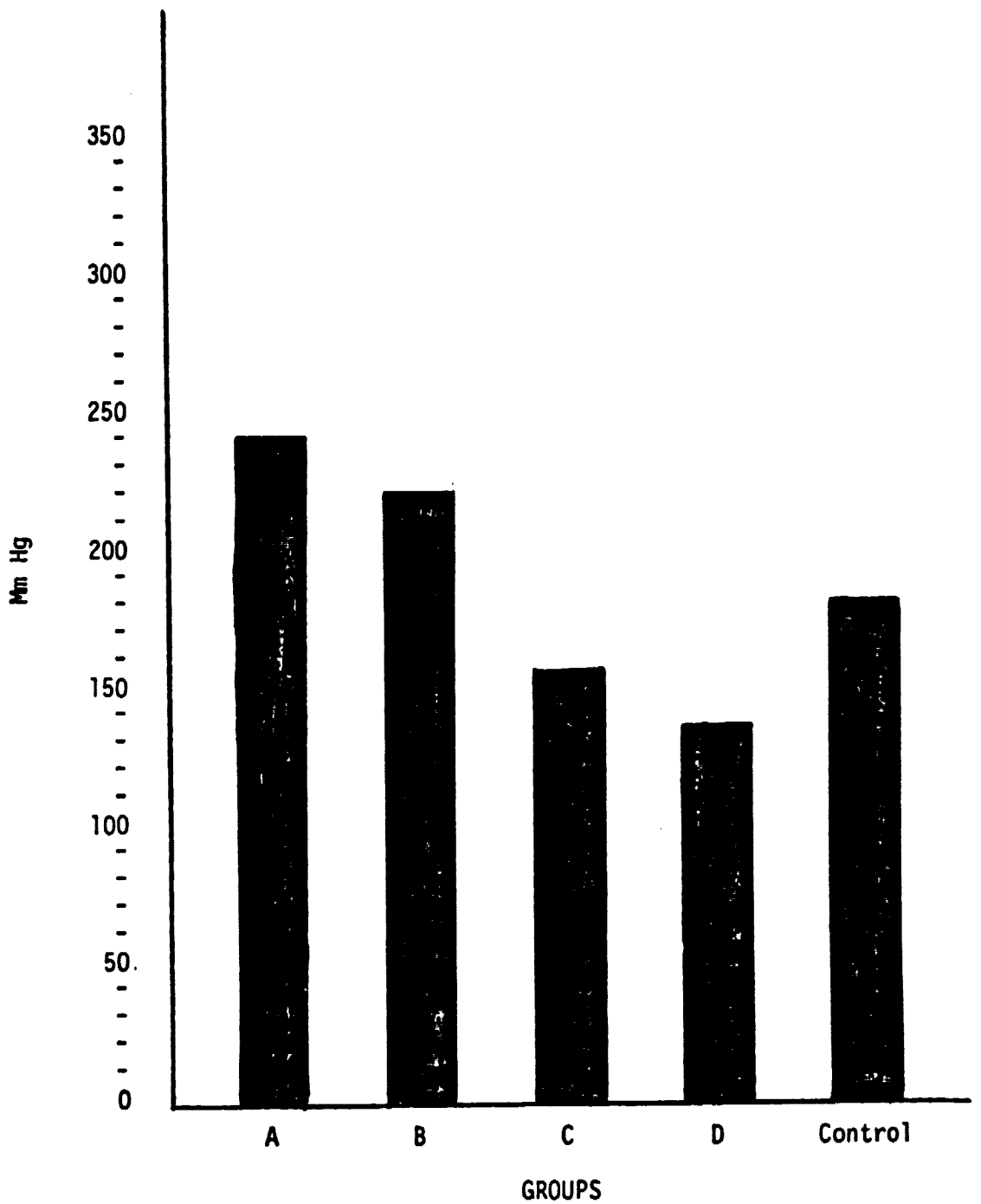


Figure II

BLOOD CLOTTING TIME

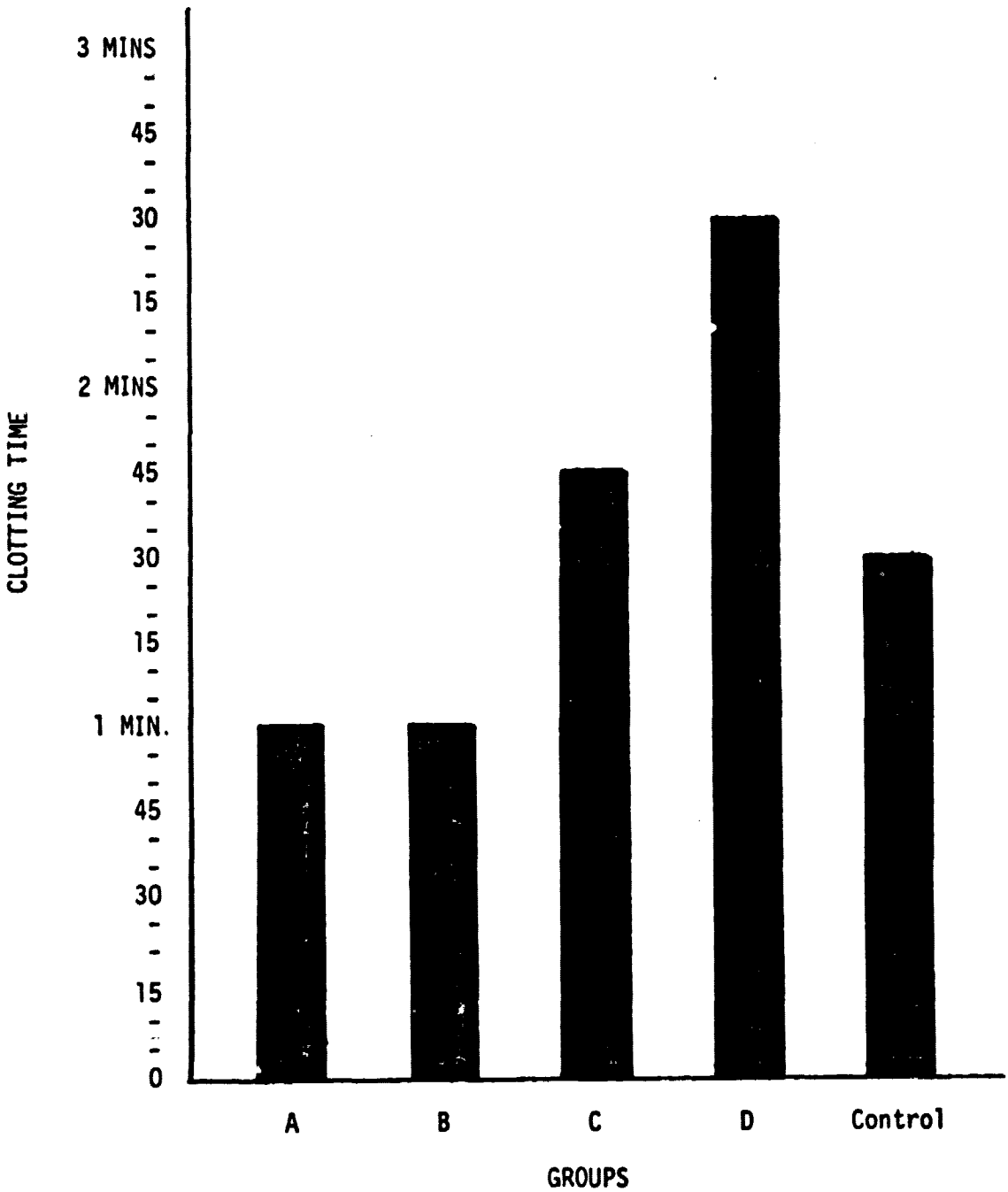
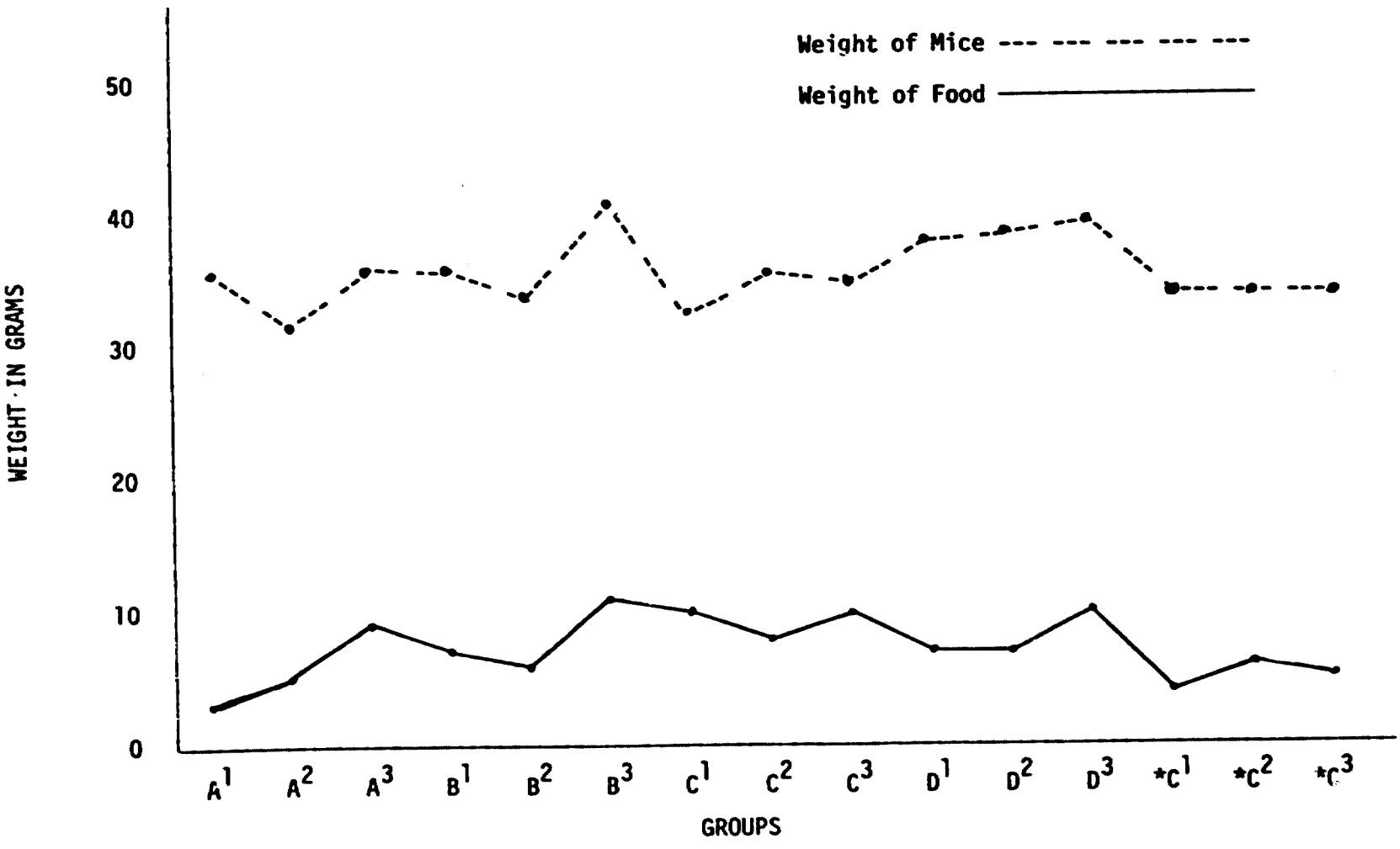


Figure III

WEIGHT OF INDIVIDUAL MICE

and

WEIGHT OF INDIVIDUAL FOOD CONSUMPTION PER DAY



*Control Group

References:

[1]Ichiharo, Ichiro. Microscopic Studies of Anterior Pituitary Glands of Vitamin E Deficient Male Mice. Nagoya, Japan: 1969.

[2]Kutsky, Roman J. Handbook of Vitamins and Hormones. New York; Nastrand Reinhold Company, 1973.

[3]Pryor, William A. Chemical and Engineering News, 34, 1971.