Effect of Vanadium on Metabolism of Glucose in the Rat

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Abstract □ A radiorespirometry experiment was conducted to determine the effects of the trace element vanadium on the metabolism of glucose-^{14}C in adult male rats by the collection of $^{14}CO_2$. Ammonium metavanadate solution was injected intraperitoneally, and labeled glucose was given *per os*. At the 0.05 significance level, an effect on metabolism was indicated by reduced levels of $^{14}CO_2$ after 1 and 3 hr. of collection but not at later times.

Keyphrases □ Vanadium—effect on glucose metabolism, rat □ Glucose metabolism—vanadium effect, rat □ Radiorespirometry—determination □ Liquid scintillation counting—analysis

Vanadium, a metallic trace element, is ubiquitous in the environment. Plants, animals, and man contain various amounts of vanadium, which has a predilection for biogenic fats and oils (1). In attempts to determine the biological functions of this element, several studies dealing primarily with the effect of vanadium on lipid metabolism were made. Bernheim and Bernheim (2), using a Warburg apparatus, demonstrated that vanadium increased the oxygen uptake of phospholipids in rat liver protein suspensions. The oxidation of other substrates including glucose, amino acids, and the lower fatty acids was not reported to be affected by vanadium.

Other investigators reported a decrease in rats in the hepatic synthesis of cholesterol (3), a decrease in the incorporation of ^{32}P into liver phospholipids (4), and a decrease in the activity of hepatic coenzyme A, coenzyme Q, and succinoxidase with vanadium present (5). The point of cholesterol inhibition has been suggested to be upon the enzyme squalene synthetase (6).

Since vanadium does affect lipid metabolism, it is reasonable to believe that the metal may also affect the metabolism of carbohydrate materials. Although the *in vitro* study of Bernheim and Bernheim (2) showed no effect of vanadium on glucose substrate, the response of the intact animal as measured with sensitive radiotracer techinques appeared to be worthy of investigation. This paper reports a study designed to determine the effects of vanadium on the metabolism of uniformly labeled glucose-^{14}C in intact rats by measuring amounts of expired $^{14}CO_2$.

EXPERIMENTAL

Radiochemical—The radiochemical purity of uniformly labeled D-glucose[1] (specific activity = 3.0 mc./mmole) was determined by paper chromatography and autoradiography. Fifteen micrograms of labeled glucose (0.25 μc.) and 10 mcg. of carrier glucose, each in a water solution, were spotted on three strips of Whatman No. 1 chromatography paper. Each strip was developed in one of three solvent systems: phenol–water (3:1 w/v), *n*-butanol–ethanol–water (52:30:18), and *n*-butanol–acetic acid–water (4:1:5). Glucose was located by lightly spraying with aniline phthalate solution[2] followed by heating 5–10 min. in an oven at 105°; brown spots formed. After development, an amount of the labeled glucose solution equal to 1% of that originally spotted was placed on each strip as a sensitivity spot. The strips were exposed to X-ray film[3] with an exposure time sufficient to allow 10^7 disintegrations from the sensitivity spots. No impurities at the 1% concentration level were revealed.

Metabolism Chambers—Four 15.24-cm. (6-in.), controlled-ventilation, glass metabolism chambers[4] were used to house the rats during CO_2 collection. A vacuum pump was used to draw CO_2-free room air through the chambers at the rate of 500 ml./min. Carbon dioxide was trapped in at least 75 ml. of a solution of 2-ethoxyethanol and 2-aminoethanol (2:1) contained in gas scrubbing towers. A secondary trap was included to ensure quantitative collection, although the efficiency of the primary trap was found to approach 100%. To prevent water quenching in the samples during counting, the chamber exhaust air was dehydrated with anhydrous calcium sulfate[5] prior to entering the primary CO_2 trapping solution.

Liquid Scintillation Counting—Activity determinations for ^{14}C were made with an internal liquid scintillation spectrometer[6] utilizing external standardization. Three-milliliter aliquots of the CO_2 trapping solution were added directly to 15 ml. of a liquid scintillator composed of 1 part toluene, 1 part 2-ethoxyethanol, and 0.8% 2,5-diphenyloxazole (8.0 g./l.). Duplicate aliquots were taken from each trap solution, and each sample was counted twice to minimize instrument error. The repeated counts on duplicate aliquots were averaged to obtain the sample activity. Primary trap samples were counted with a 1% counting error. Secondary trap samples with activities less than 1% of the primary trap activity were arbitrarily discarded. Sample counting efficiencies ranged from 70 to 73%.

Preliminary Studies—An investigation was made to determine the levels of ammonium metavanadate (NH_4VO_3) that could be safely administered to rats without causing sickness or death during the experimental period. Male rats[7] weighing approximately 200 g. were used. A single intraperitoneal dose of 11.0 mg. vanadium/kg. of body weight was found to be severely toxic, while a daily dose of 1.0 mg. vanadium/kg. was tolerated for 8 days with little observable effect. Daily doses of 2.0 and 3.0 mg. vanadium/kg. produced marked loss of weight after 7 and 5 days, respectively. As a result of these studies, single doses of 2.0 and 6.0 mg. vanadium/kg. and a daily dose of 2.0 mg. vanadium/kg. for 7 days were chosen for the respiration experiment.

Experimental Design and Procedures—The CO_2 respiration experiment was based on a completely randomized design utilizing 24 male rats weighing approximately 200 g. A control and three treatment groups, each containing six animals, were established. Four animals were tested each day for 6 consecutive days, with both treatment and day for testing assigned at random. All animals used in the respiration study were fasted for 24 hr. before dosing to reduce endogenous levels of glucose.

On the day for testing, each animal was given the appropriate amount of NH_4VO_3, dissolved in normal saline, by intraperitoneal injection followed immediately by 3.0 ml. of a 50% (w/v) glucose–water solution containing 4.01 μc./3 ml. *per os*. Control animals received labeled glucose, but saline solution was substituted for the NH_4VO_3 solution. Animals receiving repeated doses of vanadium were injected with the appropriate amount of NH_4VO_3 solution on each of 6 days prior to the respective day for testing.

Upon completion of dosing, the animals were immediately placed in the metabolism chambers where water, but no food, was pro-

[1] Amersham/Searle Corp., Des Plaines, Ill.
[2] Solution contained 0.93 g. of aniline, 1.66 g. of *o*-phthalic acid, and 100 ml. of water-saturated *n*-butanol.
[3] Kodak No-Screen Medical X-ray film, Eastman Kodak Co., Rochester, N. Y.
[4] Delmar Scientific Laboratories, Inc., Maywood, Ill.
[5] Drierite.
[6] Tri-Carb, Packard Instrument Co., Downers Grove, Ill.
[7] Sprague-Dawley descendants, Laboratory Supply Co., Indianapolis, Ind.

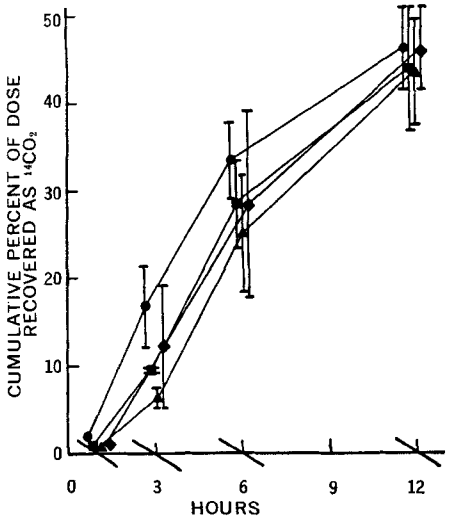

Figure 1—*Amount of $^{14}CO_2$ expired by rats receiving uniformly labeled glucose-^{14}C. Data points are staggered to include error bars which indicate 95% confidence intervals. Key: ●, control; ■, 2.0 mg. vanadium/kg.; ▲, 6.0 mg. vanadium/kg.; and ◆, 2.0 mg. vanadium/kg. daily for 7 days.*

vided. Carbon dioxide was collected for 12 hr., with the trapping solutions being changed at 1, 3, and 6 hr. At the end of the experimental period, the animals were sacrificed and discarded.

RESULTS AND CONCLUSIONS

The data from the CO_2 respiration study are shown in Fig. 1. At the end of each sampling interval, the control and the treatments were tested for significant differences at the 0.05 level by analysis of variance. Significant differences in the cumulative amounts of $^{14}CO_2$ collected were found at the 1- and 3-hr. collection intervals but not at the 6- and 12-hr. intervals. Further statistical comparison of the means by the Newman–Keul method (7), at the 0.05 level, indicated that at the 1-hr. interval each of the treatments produced significantly lower amounts of $^{14}CO_2$ than the control. At the 3-hr. interval, only the single-dose treatments were significant. Large biological variations appeared to obscure statistical significance at the 6- and 12-hr. intervals. Steadily decreasing amounts of labeled glucose in the body may also have reduced the effects of vanadium at the 12-hr. period.

Vanadium was thus found to affect glucose metabolism. The 6.0-mg. vanadium/kg. dose produced the greatest effect, but the 2.0-mg. dose also lowered $^{14}CO_2$ production significantly. Further studies are needed to determine the mode and site of action.

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