Increased Excretion of Dehydroascorbic and Diketogulonic Acids by Rats in the Cold.* (19652)

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Recent work indicates that dehydroascorbic acid (DHA) and diketogulonic acid (DKA) are not major metabolic products of ascorbic acid (AsA) breakdown in the guinea pig(1). Normally only trace amounts of DHA and DKA occur in animal tissues. When large doses of AsA are given, the increase in these oxidation products in the animal body is only a fraction of the amount which would be expected if most of the AsA were metabolized by conversion to DHA(1). The concept that AsA is utilized in the animal body as a component of some oxidation-reduction system has been frequently suggested, despite the lack of experimental evidence for employment of such a system. Numerous investigators have confirmed the fall in adrenal AsA after exposure of the intact animal to stress, following the initial reports of Sayers *et al.* on this phenomenon(2,3). Other observations have indicated AsA supplementation increases the ability of the animal to withstand stress(4), and Therien and Dugal have reported increased excretion of AsA in rats exposed to the stress of a cold environment(5).

Nearly all of the reported changes in AsA tissue levels or urinary excretion have been based on methods of analysis which do not differentiate AsA from DHA and DKA. Methods depending upon the reducing power of AsA, such as the Tillmans method and adaptations made from it(6) measure only AsA. Methods which depend upon the coupling of oxidized AsA with dinitrophenylhydrazine, such as the Roe-Kuether method(7), measure AsA, DHA, and DKA without differentiation. This report shows there is a marked change in urinary excretion of DHA and DKA, as well as of AsA, when the 3 components are determined by a method which allows analysis of each(8).

* Supported by a grant from the USAF School of Aviation Medicine, Randolph Field, Texas.

Experimental. Young albino rats, equally divided as to sex, with starting weights of about 200 g, were housed in individual metabolism cages, and fed Purina chow *ad lib.* Drinking water containing 5% ethanol as an antifreeze was provided to both control and experimental animals. The floors and funnel portions of the cages were coated with a silicone product to prevent the voided urine from coming in contact with the metal. The urine was collected into beakers containing 0.5 g $SnCl_2$ in 10% HPO_3. The cages were washed down each day and the urine and washings diluted to 200 ml with 10% HPO_3 to make a final concentration of 5% HPO_3, and analyzed for AsA, DHA, and DKA(8).

The experiments were divided into 2 series; in series I the control values were determined before the period of exposure to cold environment; in series II the controls were obtained after the experimental period. The control values were obtained while housing the animals in a room with the temperature controlled at 21°C ± 1°C, and the experimental values after placing the animals into a deep freeze box at 0°C ± 0.5°C. This box was outfitted with an intake air hose, and an outlet hose attached to negative pressure, assuring a constant flow of fresh air through the box. The excretion values for each rat were followed for about 7 days at 21°C, and 10 days at 0°C.

The animals showed great variation in daily excretion of AsA and the 2 oxidation products, both at normal and cold environmental temperatures. Table I shows the results of Series I. The excretion of AsA in the cold is 66% greater than the control excretion; that of DHA is 300% greater, and DKA is 500% increased. Table II shows the results from Series II. The values for DHA excretion did not return to control levels immediately, and the mean change in this fraction is less than in

TABLE I. Urinary Excretion of Ascorbic, Dehydroascorbic and Diketogulonic Acids by Rats at Normal and Cold Environmental Temperatures.

Fraction determined	No. of determinations 21°C	0°C	Avg 24-hr excretion in μg with S.D. 21°C	0°C	F
AsA	61	80	574 ± 661	955 ± 1235	4.79*
DHA	61	80	65 ± 66	271 ± 198	61.9 †
DKA	61	80	38 ± 62	243 ± 187	66.2 †

* Probability of variance ratio "F" less than .05, greater than .01.
† " " " " " " " .01.

TABLE II. Urinary Excretion of Ascorbic, Dehydroascorbic and Diketogulonic Acids by Rats at Cold and Normal Environmental Temperatures.

Fraction determined	No. of determinations 0°C	21°C	Avg 24-hr excretion in μg with S.D. 0°C	21°C	F
AsA	91	66	704 ± 725	500 ± 706	3.14
DHA	91	66	157 ± 170	82 ± 79	11.3 †
DKA	91	66	227 ± 181	39 ± 66	66.4 †

† Probability of variance ratio "F" less than .01.

TABLE III. Urinary Excretion of Ascorbic, Dehydroascorbic and Diketogulonic Acids by All Rats at Normal and Cold Environmental Temperatures.

Fraction determined	No. of determinations 21°C	0°C	Avg 24-hr excretion in μg with S.D. 21°C	0°C	F
AsA	127	171	536 ± 677	821 ± 995	7.75†
DHA	127	171	74 ± 72	211 ± 190	59.1 †
DKA	127	171	39 ± 63	234 ± 182	133 †

† Probability of variance ratio "F" less than .01.

Series I. In this series the AsA difference does not reach the 5% level of significance, but when both series are considered together, the greater number of degrees of freedom increases the "F" value for all fractions beyond the level of high significance, as shown in Table III. The increases for the combined values of AsA excretion in the cold average 53% more than the control excretion; DHA is 186% greater; DKA, 500% greater.

Discussion. It is interesting to speculate whether this observed increase in the excretion of oxidized AsA by the rat during exposure to low environmental temperature is the result of some mechanism in the animal body which utilizes the reducing capacity of AsA as a means of adjustment to cold stress. This is the first direct evidence that the oxidized forms of AsA may be normal metabolic products in the rat. A great many of the reported changes in AsA in the animal body might profitably be reinvestigated to ascertain whether there is a concomitant change in DHA and DKA as well.

Summary. 1. Adult albino rats were exposed to environmental temperatures of 21°C for control, and 0°C for experimental periods. 2. Urinary excretion of ascorbic acid at 0°C was increased 53%, dehydroascorbic acid increased 186%, and diketogulonic acid 500% above the values obtained on the same rats at 21°C. 3. All of these increases were found to be highly significant.

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Received June 11, 1952. P.S.E.B.M., 1952, v80.

Persistence of C^{14}-Anthranil-azo-ovalbumin in Injected Rabbits.* (19653)

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The organ deposition and intracellular distribution of heavily radio-iodinated antigens following their injection into rabbits has already been reported(1-4). Iodine 131 suffers from certain disadvantages as an antigen label in *in vivo* experiments. Iodoproteins, due to their similarity to the thyroid hormone, could conceivably be metabolized in a manner not representative of antigens in general. Moreover, the short half-life of I^{131} renders experiments of very long duration impossible. Both of these difficulties were avoided in the present investigation by coupling the protein antigen with diazotized 7-C^{14}-anthranilic acid.

*Experimental. Preparation of 7-C^{14}-anthranil-azo-ovalbumin (A*AO).* Crystalline ovalbumin was prepared from hen's eggs by the method of Kekwick and Cannan(5). All operations for the preparation of A*AO were carried out in the cold, using chilled solutions. In a typical preparation, 33 mg 7-C^{14}-anthranilic acid,§ containing 27 μc per mg, was dissolved in 2.7 ml of 0.2 *N* HCl, and diazotized by adding 0.05 *N* sodium nitrite (4.5 ml) until there developed a positive test with starch and iodide. The solution was poured into 7.5 ml of a solution containing 3.3% sodium carbonate and 3.9% ovalbumin. Coupling was accelerated by the subsequent addition of 0.95 ml *N* NaOH. After standing over night, the solution was dialyzed against distilled water, precipitated by dilute hydrochloric acid, centrifuged, and the precipitated azoprotein suspended in water and dissolved by adding sodium carbonate to pH 7. A large amount of a non-radioactive preparation was prepared parallel with the radioactive one. The 2 preparations were mixed in various ratios to afford solutions for injection of the desired specific activity. Spectrophotometric comparison of our anthranil-azo-ovalbumin with anthranil-azo-tyrosine at a wave length of 460 mμ indicated 11.8 azo groups per protein molecule, assuming a molecular weight of

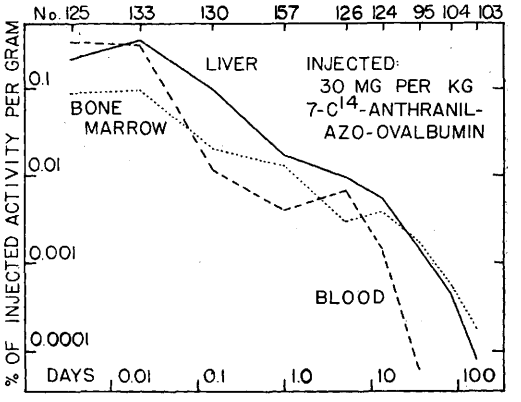

FIG. 1. Antigen content of blood, liver, and bone marrow after the injection of C^{14}-anthranil-azo-ovalbumin to rabbits. The number of each rabbit is recorded at the top of the figure; the vertical lines under the numbers indicate the time of killing; the time scale, at the bottom of the figure, is logarithmic. Analyses of other organs and of subcellular fractions of the same rabbits are recorded in Table I.

* Support of this work by the U. S. Public Health Service, National Institutes of Health, the Atomic Energy Commission, the Office of Naval Research and the American Cancer Society is gratefully acknowledged. Some of the results were presented at meeting of Am. Asso. of Immunologists, April 1952, New York City.

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§ Supplied by the Oak Ridge National Laboratories.