11. Bookman, John J., Joelson, Robert H., Toll, William G., Baker, Herman and Dolger, Henry, *ibid.*, 1957, v5, 26.

12. Mollin, D. L., and Ross, G. I. M., *The Pathophysiology of Vitamin B_{12} Deficiency in the Megaloblastic Anaemias, Vit. B_{12} and Intrinsic Factor,* 414-430, F. Enke, Stuttgart; 1957.

13. Rachmilewitz, M., Aronovitch, J., and Grossowicz, N., *J. Lab. and Clin. Med.,* 1956, v48, 339.

14. Stein, Y., Stein, Olga, Aronovitch, J., Grossowicz, N., and Rachmilewitz, M., *Bull. Research Council,* 1956, v6E, 5.

15. Beard, Marion F., Pitney, W. R., Sanneman, Everett, H., Sakal, Jarvin J., and Moorehead, Harry H., *Ann. Int. Med.,* 1954, v41, 323.

16. Beard, Marion F., Pitney, W. R., and Sanneman, E. H., *J. Hemat.,* 1954, v9, 789.

17. Erdman-Oehlecker, S., *Der Vitamin B_{12} Stoffwechsel bei Hämblastosen: Vit. B_{12} and Intrinsic Factor,* 456-472, F. Enke, Stuttgart; 1957.

18. Boger, William P., Wright, Lemuel D., Beck, George D., and Bayne, Gilbert M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 140.

19. Wright, Lemuel D., Bayne, Gilbert M., *Serum Vitamin B_{12} Concentrations of Pregnant Women and Infants, Vit. B_{12} and Intrinsic Factor,* 443-449, F. Enke, Stuttgart, 1957.

20. Malizia, E. F. del Greco, *Policlinico* (Sez prat.) 1951, v58, 1413.

21. Ralli, Elaine P., Barbosa, F. Xavier, Dumm, Mary E., Beck, Edith M., and Laken, Bertram, *J. Clin. Endocrinol.,* 1955, v15, 898.

22. Meites, Joseph, and Jeng, Y. S. L., *Fed. Proc,* 1955, v14, 100.

23. Sancetta, S. M., Ayres, P. R., and Scott, R. W., *Ann. Int. Med.,* 1951, v35, 1028.

24. Beidleman, B., *J. Clin. Nutr.,* 1953, v1, 119.

Received July 8, 1957. P.S.E.B.M., 1957, v96.

Acetoacetate Induced Dehydroascorbic Acid Accumulation in Blood and Tissues and Its Prevention by Glucose-Cyclo-Acetoacetate. (23465)

M. C. NATH AND R. M. BEHKI*
University Department of Biochemistry, Nagpur, India

Acetoacetate, an intermediary metabolite of lipids found by Nath *et al.*(1,2) to cause an immediate rise in blood glucose and decrease in glucose tolerance, has been reported(3) to cause a sudden increase in vit. C of blood of rats, when doses are relatively small. The higher doses (500 mg/kg or above) were, however, reported to depress such biosynthesis. Acetoacetate has also been reported to deplete GSH of blood of rabbits(4), and administration of the condensation product (Na salt) of glucose and acetoacetate (glucose-cyclo-acetoacetate) prior to injections of acetoacetate has been found to prevent this depletion of GSH and the rise in blood sugar(5). This depletion of GSH has been attributed to the oxidizing action of dehydroascorbic acid formed as a result of acetoacetate injections. The hydrolysed condensation product has been found by the authors(6) to act like GSH in converting dehydroascorbic acid back to ascorbic acid. It can be assumed that glucose-cyclo-acetoacetate exerts its sparing effect on GSH by undergoing enzymatic hydrolysis in the system. The present paper deals with the effect of glucose-cyclo-acetoacetate on accumulation of dehydroascorbic acid in blood and tissues and its excretion in urine as a result of acetoacetate injection in high doses.

Methods. Twenty-four male albino rats, of about 200 g each were divided into 4 groups, A, B, C, and D, of 6 rats each. Group A served as controls while Group B was given daily injection of Na-acetoacetate 500 mg/kg body weight. Group C were given daily 900 mg/kg body weight of condensation product (Na salt) prior to injections of 500 mg/kg of acetoacetate. In Group D, 900 mg/kg of hydrolysed condensation product (hydrolysed by 2N HCl for 10 minutes in boiling water, cooled and neutralized with NaOH to pH 7.2) was administered prior to acetoacetate injections daily. All injections were given intraperitoneally. The 24 hr urine of

* Government of India Senior Research Scholar.

TABLE I.* Average Urinary Excretion of Ascorbic Acid (ASA) (mg) and Dehydroascorbic Acid (DHA) per Rat per 24 Hr on:

	1st day		3rd day		7th day		12th day		19th day		24th day	
Group	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA
A	.85	.05	1.1	.09	1.15		.83	.04	1.05	.05	.95	.04
B	.53	.46	.44	.48	.38	.36	.31	.32	.36	.38	.26	.44
C	.74	.30	.88	.23	.92	.24	.78	.15	.94	.11	.88	.10
D	.92	.26	.95	.26	.92	.22	1.2	.91	.86	.26	.92	.22

* The groups are described in the text.

animals of different groups was collected in flasks containing 10 ml of metaphosphoric acid solution and the urine of each group of animals was pooled to find out the average volume of urine excreted per animal. Ascorbic acid was estimated by titrating against the standard 2:6 dichlorophenol indophenol solution and dehydroascorbic acid by passing H_2S gas and subsequent removal of excess of H_2S by CO_2 gas and titrating against the standard dye solution. Blood sugar was determined by the method of Hagedorn and Jensen(7). Rats from each group were sacrificed on the 7th, 13th, 19th and 24th day. Blood sugar, blood ascorbic and blood dehydroascorbic acids levels were determined. 10% trichloroacetic acid solution was used to deproteinize the blood and subsequent dilution was made with 6% trichloroacetic acid solution. The tissues (liver, kidney, spleen and adrenals) were macerated in sand with metaphosphoric acid-acetic acid mixtures for ascorbic and dehydroascorbic acid estimations.

Results. The 24 hour urinary excretion of ascorbic and dehydroascorbic acids of various groups estimated on different days of experiment is recorded in Table I. The levels of blood sugar and blood ascorbic and dehydroascorbic acids are summarized in Table II while the levels of the 2 acids in different tissues on 7th, 13th, 19th and 24th day have been given in Table III.

The animals given only acetoacetate (Group B) excrete more dehydroascorbic acid than the animals in other groups (Table I). Also the ascorbic acid excretion of that group is markedly less as compared to the other groups suggesting excess formation of dehydroascorbic acid. The blood sugar of Group B animals is also slightly higher than that in other groups. In the tissues of Group B animals, we find excess accumulation of dehydroascorbic acid, particularly in liver, spleen and adrenals as compared with other groups.

In Group C there is comparatively less dehydroascorbic acid and this comparison is well marked in spleen and adrenals though the livers of Group C animals do show some dehydroascorbic acid, though much less than in Group B. In the animals in Group D given hydrolysed condensation product before acetoacetate injection, dehydroascorbic acid is practically absent. The ascorbic acid contents also do not show any decrease.

Thus acetoacetate injections result in formation and accumulation of excess dehydroascorbic acid; GSH depletion following acetoacetate injections may be the result of its requirements for conversion of dehydroascorbic acid back to ascorbic acid.

Summary. 1. The effect of acetoacetate injections in doses of 500 mg/kg on ascorbic and dehydroascorbic acid contents of blood and tissues, and their excretion in urine, was

TABLE II.* Average Blood Sugar, Blood Ascorbic and Dehydroascorbic Acids on Days.

	Blood sugar, mg/100 cc blood				Ascorbic acid (reduced and oxidised) in mg/100 ml blood							
					7th		13th		19th		24th	
Group	7th	13th	19th	24th	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA
A	102.8	98.8	105.1	95.5	1.85	nil	1.92	nil	1.97	nil	1.94	.04
B	114.6	123.4	131.3	138.8	1.24	.48	1.1	.62	1.15	.75	.96	.83
C	96.8	111.4	108.8	112.4	1.7	.30	1.8	.22	2.1	.21	2.0	.19
D	109.2	107.6	111.7	113.6	2.3	.16	2.2	.21	2.1	.22	2.1	.13

* The groups are described in the text.

TABLE III.* Reduced and Oxidised Ascorbic Acid (mg) per 100 g Tissue (Liver, Kidney, Spleen and Adrenal) at Various Intervals (Days).

Group	Liver		Kidney		Spleen		Adrenal		Liver		Kidney		Spleen		Adrenal	
	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA	ASA	DHA
	7th day								13th day							
A	21.5	.21	17.5	nil	16.8	nil	267.4	1.3	20.8	.18	18.2	nil	17.1	.10	253.7	nil
B	16.9	9.5	12.8	3.2	13.3	7.9	175.5	114.8	13.1	10.3	10.1	3.3	12.4	11.6	204.2	138.8
C	22.9	3.2	19.9	2.8	17.2	1.6	320.3	17.8	18.8	5.3	19.8	2.3	19.1	.92	286.4	13.2
D	24.4	2.1	20.2	.40	15.6	1.4	328.9	nil	25.9	1.9	18.2	.24	16.1	nil	326.9	5.4
	19th day								24th day							
A	22.1	.12	17.4	.05	16.8	nil	299.8	.23	20.2	.21	18.1	nil	17.6	nil	336.8	nil
B	12.2	12.2	11.2	2.9	9.8	10.8	209.5	128.5	12.4	14.6	12.4	4.8	10.2	9.9	220.2	124.6
C	21.4	5.6	18.9	1.9	16.3	.71	340.4	8.9	20.7	4.2	20.3	2.1	17.1	.86	331.4	9.2
D	23.7	1.8	17.8	.32	16.1	nil	320.8	1.8	24.2	1.8	18.2	.21	15.9	.27	315.5	4.2

* Groups are described in the text.

studied. 2. Animals injected with acetoacetate excrete more dehydroascorbic acid and less ascorbic acid. Their tissues (liver, kidney, spleen and adrenal) also accumulate more dehydroascorbic acid, and the amount of ascorbic acid in them is diminished. 3. Glucose-cyclo-acetoacetate or its hydrolysed product has been found to prevent this increased accumulation of dehydroascorbic acid in blood and tissues. 4. Acetoacetate has been shown to cause oxidation of ascorbic acid to dehydroascorbic acid *in vitro* in presence of a few drops of $CuSO_4$ solution at a faster rate than that in the control. 5. The mechanism for prevention of acetoacetate-induced accumulation of dehydroascorbic acid by hydrolysed glucose-cyclo-acetoacetate has been suggested.

The authors are thankful to the Ministry of Education, Government of India for awarding the Government of India Senior Research Training Scholarship to one of them (R.M.B.).

1. Nath, M. C., and Brahmachari, H. D., *Nature,* 1944, v154, 487; *Ind. J. Med. Res.,* 1949, v37, 61.

2. Nath, M. C., and Chakrabarti, C. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 326.

3. Nath, M. C., Chakrabarti, C. H., Hatwalne, V. G., and Laul, K. S., *Nature,* 1948, v162, 660.

4. Nath, M. C., Hatwalne, V. G., and Gadgil, J. S., *Biochem. J.,* 1953, v53, 479.

5. Nath, M. C., and Behki, R. M., *Arch. Biochem. and Biophys.,* in press.

6. ———, *Metabolism,* 1957, v6, 127.

7. Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.,* 1923, v135, 46.

Received July 8, 1957. P.S.E.B.M., 1957, v96.