pected from different laboratories using animals from different colonies.

Summary. The intraperitoneal and intravenous routes of injection of antiserum were equally effective in development of passive anaphylactic sensitization when the guinea pigs were challenged after an incubation period of 48 hours.

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Protective Influence of Glucose Cycloacetoacetate on Glutathione Depletion in Dietary Liver Necrosis.* (26232)

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Leaf and Neuberger(1) observed that a low protein diet containing 100 mg of dry yeast per day per rat as the sole source of protein, produced an instantaneous fall in liver GSH, which after 14 days reached a constant level corresponding to 20 to 30% of the initial values. Further, this depletion of GSH was prevented by addition of cystine to the same diet. Similar results were also obtained by Lindon and Work(2) who showed that a necrogenic diet containing 18% Baker's yeast as protein source lowered liver GSH level in rats to one-fourth of its normal value in 13 days.

Recently, Nath and Behki(3) have shown that sodium salt of glucose cycloacetoacetate (GCA), a condensation product of glucose and acetoacetic ester(4) can prevent depletion of blood GSH in acetoacetate induced diabetes. The authors(5) have also observed the GSH-sparing effect of GCA in blood of scorbutic guinea pigs. It was, therefore, thought desirable to study the influence of GCA and its hydrolyzed product on depletion of GSH during feeding of necrogenic diet to rats.

Since the level of ascorbic acid in rats has also been found to be significantly depressed during development of liver necrosis (2) and since GCA has been shown to be the precursor of Vit. C in germinating legumes and in rats by Nath *et al.*(6,7), the influence of GCA and its hydrolysis product on depletion of liver ascorbic acid has also been studied. Further, in view of the protective influence of Vit. B_{12} in CCl_4 liver injury in Vit. E deficient rats(8), analogous data comprising the influence of Vit. B_{12} have been collected.

Experimental. Animals and diet: Thirty-five weanling male albino rats weighing 40-60 g were divided into 5 groups as follows: Animals in Group I were fed normal stock diet consisting of casein 12%, cane sugar 36%, arrowroot 40%, groundnut oil 6.4%, codliver oil 1.6%, salt mixture 3.5%(9), L. cystine 0.3%, choline chloride 0.2%. The following vitamins were added per kg of the

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TABLE I. Protective Influence of Vit. B_{12} and Hydrolyzed GCA on Depletion of GSH in Blood and Liver in Rats during 35 Days of Feeding Necrogenic Diet.

	Group and treatment	Blood GSH mg % ± S.D.	Blood GSH % of control	Liver GSH mg % ± S.D.	Liver GSH % of control
I	Normal stock diet (control)	33.0 ± 5.6	—	135.3 ± 17.6	—
II	Necrogenic diet	16.9 ± 2.0	51.2	88.6 ± 26.4	65.4
III	Necrogenic diet + .3% crystalline GCA in diet	17.9 ± 3.5	54.2	110.1 ± 15.2	81.3
IV	Necrogenic diet + 30 mg/kg of hydrolyzed GCA inj. on alternate days	27.2 ± 4.8	82.4	120.5 ± 11.2	89.0
V	Necrogenic diet + 200 μg/kg of vit. B_{12} inj. on alternate days	25.4 ± 4.5	76.9	127.2 ± 12.6	94.0

above diet : thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxin 5 mg, Ca-d. pantothenate 25 mg, inositol 50 mg, biotin 0.1 mg, folic acid 1 mg, Vit. B_{12} 25 μg, α-tocopheryl acetate 25 mg, Vit. K 5 mg. Animals in Group II were fed the necrogenic diet as employed by Lindon and Work(2) modified as follows : Baker's yeast 6%, cane sugar 36.5%, arrowroot 46%, codliver oil 1.6%, groundnut oil 6.4% and salt mixture 3.5%. Animals of Groups III, IV and V were kept on the same necrogenic diet as in Group II animals. Group III animals were given, along with their diet, crystalline GCA at 0.3% level. Group IV animals were injected subcutaneously, on alternate days, with 30 mg equivalent hydrolyzed product of GCA/kg body wt. Group V animals were injected subcutaneously with 200 μg/kg body wt. of Vit. B_{12} (B-Douza, Lab. Grimault, Paris). All the animals were fed the diets and water *ad libitum*.

Analytical methods. GCA was prepared according to the method of West(4). Hydrolyzed product of GCA was prepared as follows: In a 50 cc conical flask 2 g of crystalline GCA was hydrolyzed with 10 cc 2N HCl over a boiling water bath for 20 minutes. On cooling, the contents of the flask were neutralized and extracted 3 times with ether discarding the upper ethereal layer. Air was bubbled through the aqueous extract until free from ether. Its final pH was adjusted to 7.2 with 0.1 N NaOH.

The rats were killed by a blow on the head and immediately after veni-puncture, the blood was collected in an oxalated container and its glutathione content determined by the procedure of Woodward and Fry(10). After taking blood, a few pieces of liver (about 1 g) were cut out, dried on filter paper and dropped quickly into a weighed beaker containing about 10 cc of 2% sulphosalicylic acid solution. Ascorbic acid was determined by titration with indophenol and GSH was estimated according to a method of Woodward and Fry(10) modified by Lindon and Work(2).

Results and discussion. During 5 weeks of feeding necrogenic diet, liver and blood GSH levels were found to be reduced to 65% and 51% respectively of the average values for normal animals (Table I). The fall in level of blood GSH can be compared to that recorded by Lindon and Work(2) who observed a fall in blood GSH to 68% of initial value in 13 days. However, the reduction in liver GSH to 65% of average normal value as recorded by us, deviates considerably from the results of Leaf and Neuberger (1) and Lindon and Work(2), who recorded a reduction of GSH levels to 20-30% of controls. The disparity in our results can be ascribed mainly to 2 factors, *viz.* the quality of yeast employed as a source of protein in the diet and the delay in feeding necrogenic diet during the weanling stage. The importance of these 2 factors in liver necrosis has been demonstrated by Naftalin(11).

Table I shows that levels of liver GSH in rats (fed on necrogenic diet only) were lowered to 65.4% of normal controls whereas in the animals injected with Vit. B_{12} and hydrolyzed GCA, corresponding values of GSH re-

TABLE II. Sparing of Ascorbic Acid by Hydrolyzed GCA and GCA in Rats Fed on Necrogenic Diet for 35 Days.

Group and treatment		Liver ascorbic acid mg % ± S.D.	% of control
I	Normal stock diet	24.7 ± 3.8	—
II	Necrogenic diet	15.7 ± 4.3	63.5
III	Necrogenic diet + 0.3% crystalline GCA in diet	22.1 ± 1.9	89.4
IV	Necrogenic diet + 30 mg/kg body wt of hydrolyzed GCA inj. on alternate days	23.0 ± 2.6	93.1
V	Necrogenic diet + 200 μg of vit. B_{12} inj. on alternate days	20.9 ± 2.7	84.6

duction were 94% and 89% of controls respectively, showing a marked GSH-sparing influence of hydrolyzed GCA.

Crystalline GCA administered orally has been found to be less active than the hydrolyzed product, probably because of poor absorption of GCA through gastrointestinal tract. Nath and Saikia(12) have observed that methionine content of the liver in rats injected with hydrolyzed GCA is considerably higher than that of animals fed on high protein diet during development of experimental atherosclerosis. GSH-sparing influence of hydrolyzed product of GCA may, as such, be explained broadly by virtue of its ability to spare methionine, which has been shown by Leaf and Neuberger(1) to check depletion if liver GSH.

During feeding of necrogenic diet level of liver ascorbic acid was also lowered to 63.5% of normal control animals (Table II). A similar observation was also recorded by Lindon and Work(2). It was interesting to find that both GCA and its hydrolyzed product checked depletion of ascorbic acid considerably. Vit. B_{12} was inferior in this respect. Sparing of liver ascorbic acid by GCA may possibly be explained by the role of GCA as precursor in biosynthesis of Vit. C as shown by Nath and coworkers(6,7) in germinating legumes and in rats.

Summary. The influence of glucose cycloacetoacetate (GCA), its hydrolysis product and Vit. B_{12} on depletion of blood and liver glutathione and liver ascorbic acid in rats during 35 days of feeding necrogenic diet containing 6% baker's yeast as the sole protein source, was studied. GSH level in blood and liver in animals receiving necrogenic diet was reduced to 51% and 65% of normal controls, whereas in Vit. B_{12} injected animals corresponding values were 89% and 94% and in animals injected with hydrolyzed GCA respective values were 82% and 99%. Liver ascorbic acid of the animals fed on necrogenic diet was reduced to 63% of controls, while that in the animals injected with Vit. B_{12} and with hydrolyzed GCA it was recorded to be 93% and 84% of controls respectively.

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