Although the pituitary-adrenal axis has been implicated in the development of PNMT activity in the fetus(8), the mechanism of action of dexamethasone in causing elevation in enzyme activity in the 21-day-old animal as well as the control of PNMT activity by 45 days requires elucidation. Furthermore additional work is necessary to describe factors other than PNMT that contribute to the observed increase in adrenal epinephrine levels. These might include the activity of catecholamine-synthesizing and degradative enzymes, and the binding characteristics of endogenous epinephrine. Such studies are presently underway in this laboratory.

Epinephrine is involved in a wide variety of biological functions. The finding that treatment of pregnant rats with steroids leads to enhanced levels of epinephrine in the progeny suggests an animal model for further studies of the physiological and psychological (10) role of high endogenous levels of this catecholamine in mammals.

Summary. The administration of the synthetic glucocorticoid dexamethasone to pregnant rats raised the activity of the adrenal epinephrine-forming enzyme (PNMT) in the 21-day-old offspring. Adrenal epinephrine content was also elevated at 21 and 45 days. Heart PNMT activity was raised in the immediate postnatal period in the progeny of steroid-treated mothers, although enzyme activity returned to control values at 21 days. Brain PNMT activity was unaffected by steroid treatment.

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Disturbance of Cholesterol Metabolism in Alloxan Diabetes and Its Prevention by Glucose-cycloacetoacetate. (32558)

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Best(1) is of the opinion that the incidence of atherosclerosis is high in diabetes. Root and Wilson(2) have reported that a close correlation exists between diabetes and atherosclerosis. Our experimental work supports such a view in that continued injection of acetoacetate not only produces hyperglycemia (3) but also tends to lead to thrombosis(4).

The present investigation is based on the report of several workers that cholesterol and other lipids tend to be high in diabetes (5-8) and our own finding that the onset of diabetes caused by alloxan can be prevented by glucose-cycloacetoacetate, GCA(9-12). GCA is a crystalline compound formed by condensing glucose with acetoacetate. The purpose was to determine the C/P ratio (total cholesterol/lipid phosphorus) in plasma and tissues of alloxan diabetic rats and to see whether GCA can effectively maintain this ratio within normal limits. Furthermore, Nath and Brahmankar(13) have shown that GCA can increase elimination of fecal bile acids of animals fed a high saturated fat diet. Siperstein *et al*(14) have reported that excess cholesterol is metabolized and eliminated as

fecal bile acids. Thus, it was surmised that if the metabolism of cholesterol suffers in diabetes leading to a reduction in the amount of bile acids, GCA may be helpful in restoring metabolism of cholesterol and increasing the elimination of bile acids in the feces.

Materials and methods. 24 adult male albino rats weighing between 150-200 g were given the basal diet of the following composition: wheat flour 60%, yeast 10%, groundnut oil 10%, casein 15%, salt mixture (Hawk Oster) 5%. Crystalline GCA was prepared by condensing glucose with ethyl acetoacetate by the method of West(15) as modified by Nath *et al*(16). The sodium salt of GCA was prepared by suspending an accurately weighed quantity of GCA (2.74 g) in a solution of NaOH (0.4 g in 20 ml) and heating the mixture in a boiling waterbath. This was then cooled, adjusted to pH 7.2 with 0.1 N HCl and diluted with distilled water so that each ml contained 100 mg of the compound.

The rats were equally divided in 3 groups. Group I animals were treated as controls. Group II animals were made diabetic by injecting alloxan monohydrate, 20 mg/100 g of body weight, subcutaneously for 2 consecutive days. Animals of Group III were injected subcutaneously with Na-GCA 45 minutes prior to injection of the diabetogenic dose of alloxan in molar ratio of 3:1 of Na-GCA/alloxan for 2 days. Injection of Na-GCA was repeated for 5 days more.

Blood was collected by cutting the tails periodically and blood sugar estimated according to the method of Nelson(17). After 14 days, 24 hour-feces of all the animals, which were kept in the metabolism cages, individually, were collected and bile acids estimated according to the method given in Colorimetric Methods of Analysis(18) using levulose as coloring reagent.

The animals were then sacrificed and cholesterol levels of the plasma, liver and kidney were determined by the method of Abell(19). Lipid phosphorus contents of plasma, liver and kidney were determined by the method of Youngberg and Youngberg(20), using the phosphate procedure of Fiske & Subbarow (21).

Results and discussion. It is evident from Table I that all the animals pretreated with Na-GCA before alloxan injection show normal blood sugar values. The excretion of bile acids is low in alloxan-induced diabetic animals, whereas the excretion of bile acids is found to be nearly normal in animals treated with Na-GCA.

TABLE I. Excretion of Fecal Bile Acids in Alloxan Diabetes and the Effect of Na-GCA.

Groups	Treatment	Fecal bile acids in mg/100 g of feces ± S.D.	Blood sugar level in mg/100 ml of blood ± S.D.
I	Control (6)	534 ± 53	99.2 ± 3.4
II	Alloxan (7)	198 ± 84	446.6 ±20.5
III	Na-GCA + alloxan (6)	396 ± 66	100.4 ± 4.5

Figures in parentheses indicate number of animals used.

Table II shows that there is an enormous rise in cholesterol content of liver and kidney of alloxan diabetic rats. The lipid phosphorus contents however increase in some cases but

TABLE II. C/P Ratio in Plasma, Liver and Kidney in Alloxan Diabetes and Effect of Na-GCA.

Group	Tissue	Treatment	Total cholesterol, mg/100 ml or 100 g ± S.D.	Lipid phosphorus, mg/100 ml or 100 g ± S.D.	C.P. ratio
I	Plasma	Control (6)	101.6 ± 20.5	5.1 ± 1.0	20.0
II	"	Alloxan (7)	168.4 ± 26.5	5.2 ± 2.5	32.6
III	"	Na-GCA + alloxan (6)	124.0 ± 50.9	5.7 ± 0.4	21.8
I	Liver	Control (6)	299.4 ± 53.6	59.3 ± 15.4	5.0
II	"	Alloxan (7)	515.0 ± 42.4	50.1 ± 15.3	10.1
III	"	Na-GCA + alloxan (6)	447.2 ± 24.9	56.2 ± 4.6	8.0
I	Kidney	Control (6)	457.0 ± 64.2	58.0 ± 26.8	7.8
II	"	Alloxan (7)	741.5 ±107.0	66.5 ± 22.8	11.2
III	"	Na-GCA + alloxan (6)	602.4 ± 48.7	77.2 ± 10.3	7.8

Figures in parentheses indicate number of animals sacrificed.

not to a great extent, thus resulting in the elevation of the C/P ratio. But when the sodium salt of GCA was administered 45 minutes prior to the injection of the diabetogenic dose of alloxan, cholesterol contents in blood and tissues are much lower than in those of the diabetic animals and the lipid phosphorus values are increased. This brought down the C/P ratio to a considerable extent in all such cases.

Gordon *et al*(22) have reported an increase in elimination of fecal bile acids with a concomitant fall in serum cholesterol levels when sunflower seed oil was substituted for coconut oil in the diet. Our previous findings(13) as well as the results of the present investigation suggest the possibility that one mechanism by which substances such as Na-GCA bring about a reduction in cholesterol levels of plasma and tissue is through increased excretion of bile acids, the end product of cholesterol metabolism.

Summary. The effect of Na-GCA on total plasma and tissue cholesterol levels in excretion of bile acids in the feces has been studied in rats with diabetes induced by injections of alloxan. The increase in the C/P ratio in plasma, liver and kidney produced by the alloxan is antagonized by Na-GCA if the latter compound is administered before the alloxan. Na-GCA increases output of bile acids in feces and concomitantly decreases the total plasma and tissue cholesterol content. Thus it appears that GCA increases metabolism of cholesterol to bile acids in the system studied.

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