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Liver Ketogenesis. II. Role of Glucose Cycloacetoacetate on Ketogenesis in Hyper- and Hypothyroid Rats.* (25595)

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The authors reported that hydrolyzed glucose cycloacetoacetate (GCA) when added to liver slices in presence of pyruvate and acetate enormously decreases formation of acetoacetate(1) and increases glycogen(2). Because thyroid feeding (reviewed by Barker(3)) causes enormous rise in production of acetoacetate in the liver, we attempted to find out the role of hydrolyzed GCA in preventing excess of ketogenesis in liver induced by thyroid feeding. We also studied the effect of thiouracil and the combined effect of thiouracil and GCA on acetoacetate formation from lower fatty acids in presence of liver slices. Utilization of acetoacetate by kidney slices in different groups of animals has also been measured.

Method. In view of relatively rapid response of young rats to thyroid and thiouracil feeding(4), male albino rats weighing 75 g to 100 g and growing on standard diet, were used. The animals were divided into 6 groups of 6 rats each and fed the following basal diet *ad lib.* for 6 weeks:

	%		%
Wheat flour	10	Salt mixture	5
Sucrose	49	Yeast powder	4
Casein	20	Marmite	2
Groundnut oil	10	Cod liver oil	.5

Rats in Group I received basal diet and Group II basal diet plus freshly hydrolyzed GCA, 0.8% of diet by weight. Desiccated thyroid (0.3% of diet by wt) was mixed in diet of animals in Groups III and V and thiouracil 0.3% in Groups IV and VI as these concentrations were effective(Hander,5). Rats in Groups V and VI received 0.8% GCA hydrolyzed daily in addition to thyroid and thiouracil respectively.

Tissue preparation and analytical methods. At the end of 6 weeks, animals were stunned by a blow on head and decapitated. Liver and kidneys were taken out immediately and placed in ice-cold saline. Tissue preparations were done as described by Umbreit *et al.*(6). Liver and kidney slices were cut with razor blade, suspended in conventional Warburg vessels containing 3 cc of Krebs' Ringer Phosphate buffer of pH 7.2. The vessels were gassed for 1 minute with oxygen, then incubated for 2 hrs. at 37.5°C with constant shaking. Then acetoacetate was estimated from media in vessel according to manometric method of Edson(7). Liver glycogen estimations were done by the method of Good *et al.* (8) and blood sugar by that of Hagedorn and Jensen(9). GCA, a crystalline product obtained by condensation of glucose with acetoacetate, was prepared according to the method of West(10) modified by Nath *et al.*(11). It was hydrolyzed with 2N HCl for 12 min and extracted with ether to remove resinous materials formed. Aqueous solution was adjusted to pH 7.2 by adding requisite amount of 2N NaOH, then fed to the animals. Total fat content of liver was extracted thrice with alcohol-ether mixture (1:1). The extract was

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TABLE I. Body Weight Gain, g/100 g/Week, of Different Groups of Rats.

Group	No. of animals	Period in wk 1	2	3	4	5	6	Avg food intake, g/rat/day
Normal	6	8	16.1	24.4	32.2	37.7	45.2	10
Hydrolyzed GCA fed	6	7	13.2	25.2	37.7	47.7	55.2	10
Thyroid fed	5	6	12	17.6	19.6	21.6	21.6	15
Thiouracil fed	6	7	14	20	25	27.5	30	8
Thyroid + hydrolyzed GCA	6	5	10	14.6	19.1	20.9	21	12.5
Thiouracil + hydrolyzed GCA	6	5	10	14.6	18.6	22.9	24.9	8

evaporated to dryness and the remaining solid reextracted with petroleum ether (40-60° B.P.). Petroleum ether was evaporated to dryness and amount of dry fat calculated. At end of every week, rats were weighed and average food consumption in g/day/rat was calculated. Urine was tested qualitatively for sugar and ketone bodies.

Results. Blood sugar values (Table II) indicate that hydrolyzed GCA feeding to hyper- and hypothyroid rats, Groups V and VI ($t = 2.3$ and 1.7 respectively) has a hypoglycemic effect when compared to respective control Groups III and IV. In normal rats, however, it does not seem to affect blood sugar level significantly ($t = 1.05$).

Carcass lipids(12) and liver glycogen are very low in hyperthyroid animals(13, 14) but feeding of hydrolyzed GCA to Group V causes a significant increase in liver glycogen as compared to Groups III, IV & VI. Beneficial effect of GCA on glycogenesis in liver and diaphragm has already been reported(2). Feeding of hydrolyzed GCA to normal animals (Group II) increases total fats in liver to some extent as compared with normal (Group I).

Gross enlargement of liver and kidney in hyperthyroid animals agrees with observations of other workers(15,16) and hydrolyzed GCA feeding to these rats (Group V) prevents such enlargement. Hypertrophy of kidney in hypothyroid rats is also prevented by feeding hydrolyzed GCA (Group VI).

Table III indicates oxygen consumption and acetoacetate production by the liver slices from different groups. That general metabolism is accelerated considerably in hyperthyroid animals has already been shown(3). Increased oxygen consumption and acetoacetate production in Group III confirm these findings. In this group, liver produces large amounts of acetoacetate though there is some increase in utilization by kidney slices (Table IV). Acetoacetate produced in Group II is significantly less than that in normal rats (Group I) and hydrolyzed GCA feeding prevents excess of ketogenesis by liver slices as seen by comparing results of Groups III and V. Endogenous acetoacetate production in kidney is high in hypothyroid rats as observed by Mookerjea and Sadhu(17). Lower rate of oxygen consumption and acetoacetate production in liver of hypothyroid rats indicates slowing of metabolism. Utilization of acetoacetate by kidney slices of animals fed thy-

TABLE II. Blood Sugar, Liver Glycogen, Liver Fat, % of Tissue Weights (Wet Wt) of Different Groups of Rats.

Group	No. of animals	Blood sugar, mg/100 cc	Liver glycogen, g/100 g	Liver fat, g/100 g	Wet wt of tissue, g/100 g body wt Liver	Kidneys
Normal	6	86 ± 2	2.18 ± .2	3.5 ± .3	3.6 ± .18	.82 ± .04
Hydrolyzed GCA fed	6	78 ± 3.5	2.14 ± .14	4.04 ± .28	3.61 ± .13	.70 ± .03
Thyroid fed	5	100 ± 3	.21 ± .10	4.3 ± .31	5.2 ± .2	1.10 ± .05
Thiouracil fed	6	90 ± 2	3.0 ± .25	4.0 ± .28	4.01 ± .1	.92 ± .04
Thyroid + hydrolyzed GCA	6	83 ± 4	1.65 ± .21	4.4 ± .21	3.43 ± .11	.96 ± .03
Thiouracil + hydrolyzed GCA	6	80 ± 3	3.6 ± .2	4.4 ± .23	4.0 ± .12	.82 ± .04

TABLE III. Oxygen Uptake and Acetoacetate Production by Liver Slices from Different Groups of Animals.

Group	No. of animals	Oxygen uptake in μl/100 mg wet wt of liver slices in 2 hr			Acetoacetate produced in *μl/100 mg of liver slices, wet wt, in 2 hr		
		No substrate	Butyrate, .017M	Acetate, .08M	No substrate	Butyrate, .017M	Acetate, .08M
Normal	6	230 ± 12	354 ± 15	276 ± 20	15 ± 6	85 ± 10	60 ± 13
Hydrolyzed GCA fed	6	240 ± 15	365 ± 17	265 ± 19	12 ± 7	81 ± 11	38 ± 13
Thyroid fed	5	280 ± 12	400 ± 20	338 ± 18	65 ±15	240 ± 16	280 ± 25
Thiouracil fed	6	153 ± 10	251 ± 15	190 ± 12	30 ±11	46 ± 12	33 ± 6
Thyroid + hydrolyzed GCA	6	209 ± 15	386 ± 20	283 ± 21	29 ± 7	102 ± 20	67 ± 11
Thiouracil + hydrolyzed GCA	6	158 ± 11	190 ± 10	162 ± 9	6 ± 4	46 ± 5	15 ± 6

Krebs' Ringer phosphate buffer pH 7.2; incubation temp. 37.5°C; oxygen as gas phase.

* CO_2 in μl produced after decarboxylation of acetoacetate with aniline citrate.

roid and thiouracil respectively along with hydrolyzed GCA (Groups V and VI) is considerably increased.

Traces of sugar and ketone bodies were found in urine of animals after feeding thyroid for 3 weeks (Group III). Urine of other groups was free from sugar and ketone bodies.

Discussion. Results indicate acceleration of metabolic process in hyperthyroid and reduction in hypothyroid rats as previously described(3). Polyphagia and inhibition of gain in weight in hyperthyroidism, is known(18) so also the effect of thiouracil *i.e.,* lower metabolic rate and retardation of growth as described by Astwood *et al.*(19,20). Higher rates of acetoacetate production, its utilization and oxygen consumption found in hyperthyroid rats can be attributed to increase in metabolism, while the reverse is true of hypothyroidism. However, increased rate of endogenous acetoacetate production by kidney slices of hypothyroid animals, also observed by Mookerjea *et al.*(17), cannot be explained satisfactorily.

The significant effect of hydrolyzed GCA in preventing ketogenesis and accelerating acetoacetate utilization by kidney slices in different groups is evident from our results.

Ketogenesis is intimately related to lipogenesis and glycolysis. Thus Lyon *et al.*(21) showed that ketogenesis is inversely proportional to long chain fatty acid synthesis.

Liver slices in presence of hydrolyzed GCA synthesized fat from acetate at a higher rate than in control *i.e.,* without hydrolyzed GCA (unpublished observation). Thus, there is every possibility of hydrolyzed GCA acting to divert the acetyl-CoA, towards lipid synthesis, thereby reducing amount available for its condensation to form acetoacetate.

Summary. Feeding of hydrolyzed glucose cycloacetoacetate (GCA) promotes growth in normal animals and prevents excess of acetoacetate production by liver slices in normal as well as hyperthyroid rats. It accelerates acetoacetate utilization by kidney slices in

TABLE IV. Acetoacetate Used by Kidney Slices. Krebs' Ringer phosphate buffer pH 7.2; incubation temp. 37.5°C; oxygen as gas phase.

Group	No. of animals	Oxygen uptake, μl/100 mg kidney slices wet wt of tissues in 2 hr		Acetoacetate, μl/100 mg/2 hr by kidney slices	
		No substrate	Acetoacetate, 300 μl	Produced (without substrate)	Used 300 μl acetoacetate
Normal	6	560 ± 20	687 ± 31	Negligible	84 ± 14
Hydrolyzed GCA fed	6	514 ± 16	762 ± 17	"	185 ± 19
Thyroid fed	5	658 ± 16	917 ± 30	15 ± 4	118 ± 15
Thiouracil fed	6	459 ± 17	530 ± 18	33 ± 4	58 ± 15
Thyroid + hydrolyzed GCA fed	6	620 ± 20	731 ± 28	12 ± 3	147 ± 25
Thiouracil + hydrolyzed GCA fed	6	530 ± 12	630 ± 18	8 ± 3	90 ± 7

normal as well as in hyper- and hypothyroid rats. Higher percentage of liver glycogen and lower blood sugar was found in animals fed with hydrolyzed GCA, as compared to their appropriate controls.

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Urinary Excretion of Acid Mucopolysaccharides by Rabbits Injected with Chelating Agents and with Chondroitin Sulfate.*† (25596)

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Of recent years there has been interest in treatment of scleroderma by chelation(1-4). The therapeutic efficacy of this approach is yet to be established. However the possibility of connective tissue being changed by chelators is intriguing. The present pilot study was undertaken in rabbits prior to studying humans to see if a change in excretion of acid mucopolysaccharides in urine could be detected following administration of chelating agents. Bryant, Leder and Stetten(5) have shown quantitation of urinary acid mucopolysaccharides to be a potentially rewarding approach to reflection of experimental alteration of connective tissue in rabbits. as demonstrated by their data on urinary levels following injection of papain intravenously. Their observations convey the implicit assumption that proteolytic enzyme as administered could affect connective tissue extravascularly and that chondroitin sulfate presumably released from protein binding *in situ* could then pass intravascularly to the kidneys where it could be excreted at least in part in the urine. In the present study it is shown that chondroitin sulfate† injected in large amounts intravenously into rabbits resulted in excretion of a small proportion but nonetheless a large amount of chondroitin sulfate in urine. When chelators‡ were administered extravascularly to rabbits, a transient and frequently mild rise in urinary excretion of acid mucopolysaccharides was noted; time of onset and degree and

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† Commercial chondroitin sulfate (General Biochemicals) of cartilaginous source, containing 15 to 20% protein.

‡ Disodium EDTA, with and without calcium furnished kindly as Endrate by Abbott Research Labs., North Chicago, Ill.