Liver Ketogenesis. IV. Ketogenesis in Thiamine Deficient Rats and Effect of Hydrolyzed Glucose Cycloacetoacetate Feeding.* (26468)

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Thiamin deficiency is known to cause disturbances in carbohydrate metabolism such as hyperglycemia, reduced sugar tolerance and rise in blood pyruvate(1,2). The authors have reported that glucose cycloacetoacetate (GCA) a crystalline product obtained by condensation of glucose with acetoacetate, increases pyruvate utilization by rat liver slices and diaphragm *in vitro*(3). GCA (hydrolyzed) was also found to be antiketogenic *in vitro* and *in vivo*(4). In view of these findings, our study of the effect of hydrolyzed GCA or GCA (h) feeding on pyruvemia in B_1 deficiency is reported here. Liver ketogenesis and acetoacetate utilization by kidney slices and diaphragm of rats given B_1 deficient diet for a period of 4 weeks were studied. The effect of GCA (h) feeding along with deficient diet is also reported.

Method. Male albino rats growing steadily on standard diet were divided into 4 groups. Group I was given normal diet along with thiamin. Group II was fed B_1 deficient diet, the composition of which is given below. Group III received .8% GCA (h) (of diet by wt.) along with deficient diet. Because B_1 deficient rats are known to eat less, Group IV, which received the measured amount of diet equivalent to that consumed by Group II, was included.

Composition of diet	%
Starch	68
Vitamin free casein	20
Groundnut Oil	5
Salt Mixture	4
Cod liver oil	2

Vitamin mixture added mg/kg of above diet every day: Riboflavin, 4, Pyridoxine 2, Nicotinic acid 25, Cal. pantothenate 20, choline chloride 1000, Biotin .1, Folic Acid 2, Vit. B_{12} .03, Vit. K 10, Vit. E 25. Thiamin, 50 mg/kg was added to the diet fed to Groups I and IV.

GCA was prepared by the method given by West(5) as modified by Nath *et al.*(6). It was hydrolyzed in 2N HCl for 12 min in boiling water bath. Other details have been published(3). At the end of 4th week feeding period, when rats were expected to develop B_1 deficiency as confirmed by steady weight loss and reduced food consumption, they were sacrificed by decapitation. The following estimations were made: Blood sugar by Hagedorn and Jensen method(7), blood pyruvate by Friedman(8) and liver glycogen by that of Good *et al.*(9). Wet weight of total liver, kidneys and adrenals was recorded. Tissue preparation for *in vitro* work was done by the method of Umbreit *et al.*(10). Liver and kidney slices were cut free-hand using razor blade and suspended in 3 ml of Krebs Ringer phosphate buffer, pH 7.2 with appropriate substrate. Incubation was carried out in conventional Warburg vessels for 2 hours at 37.5°C with constant shaking. At the end of incubation period, acetoacetate in media was estimated by menometric method of Edson(11).

Results and discussion. Hyperglycemia and pyruvemia in B_1 deficient rats (Group II) as compared to normals as well as controls (Group IV) are shown in Table I. GCA (h) feeding to B_1 deficient rats restores these values to normal. Our previous finding(3) that GCA (h) increases pyruvate utilization *in vitro* is thus substantiated. Thiamine is reported to be essential for lipogenesis(12). Liver glycogen and lipids are significantly diminished in B_1 deficiency (Group II) but only slightly in Group IV, *i.e.*, rats receiving restricted amount of diet. This indicates that besides inanition, B_1 deficiency has depressing effect on liver glycogen and lipid content. We have reported earlier that GCA (h) increases glycogen synthesis in liver slices and diaphragm(13). Chronic under-nutrition ex-

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TABLE I. Blood Sugar, Blood Pyruvate, Liver Glycogen and Lipids, % of Tissues Weights (Wet Wt) and Acetoacetate Production by Liver Slices and Its Utilization by Diaphragm and Kidney Slices.

Group	I	II	III	IV
No. of rats	8	12	8	6
Blood				
Sugar, mg/100 cc	90 ±3	105 ±3	91 ±4	92 ±4
Pyruvate, mg/100 cc	.86 ± .12	5.0 ± .3	1.13 ± .2	.91 ± .16
Liver				
Glycogen, g/100 g wet wt of tissue	3.3 ± .16	2.0 ± .18	2.8 ± .21	3.1 ± .2
Lipids, *idem*	4.8 ± .5	3.4 ± .3	4.4 ± .3	4.2 ± .4
Tissue wt				
Liver, g/100 g wet wt of tissue	3.5 ± .1	4.3 ± .13	4.0 ± .1	3.8 ± .15
Kidney, *idem*	.23 ± .03	1.16 ± .05	.8 ± .04	.81 ± .05
Adrenals, mg/100 g wet wt tissue	12 ±1	18 ±2.5	11.8 ±2	14 ±2
Acetoacetate produced,* μl/100 mg of liver slices				
Butyrate, .017 M	66 ±5	100 ±8	69 ±6	73 ±7
Acetate, .08 M	50 ±4	80 ±7	55 ±5	60 ±6
Acetoacetate utilized† in μl/100 mg wet wt of tissue				
Kidney slices	68 ±5	51 ±4	69 ±6	58 ±5
Diaphragm	45 ±3	15 ±4	40 ±5	35 ±6

* μl CO_2 produced after decarboxylation of acetoacetate with aniline citrate.
† 150 μl acetoacetate added as substrate.
Krebs' Ringer phosphate buffer, pH 7.2, temp. 37.5°C. Incubation period 2 hr. Air as gas phase.

erts a non-specific stress and causes hypertrophy of adrenals(14) but this hypertrophy is also present in B_1 deficiency even when evaluated with pair feeding technic(15). Hypertrophy of tissues, especially of adrenals, in B_1 deficiency is also shown in our results (Table I). GCA (h) feeding has been found to maintain normal adrenal weight in deficient animals.

Acetoacetate production by liver slices is considerably increased in B_1 deficiency and its utilization by diaphragm and kidney slices is decreased. Liver glycogen has been known to influence lipogenesis and ketogenesis(16). Mirsky(17) has shown that depletion of liver glycogen causes greater oxidation of fatty acids and greater production of ketone bodies. It may be assumed that lower levels of liver glycogen in B_1 deficient rats coupled with decreased lipogenesis by adipose tissue, accelerate ketogenesis by liver slices. GCA (h) feeding increases acetoacetate utilization and prevents its excessive production.

Beatty *et al.*(18) have reported that glucose has no effect on acetoacetate utilization in the muscle and diaphragm *in vitro* but GCA, which on hydrolysis gives rise to a enediol glucose(19) has great effect in accelerating acetoacetate utilization.

Summary. B_1 deficiency in rats causes hyperglycemia, pyruvemia and reduces liver glycogen and total lipids. Marked hypertrophy of tissues especially of adrenals is also found. Acetoacetate production by liver slices is increased and its utilization by kidney slices and diaphragm decreased. Beneficial effects of GCA (h) feeding to B_1 deficient rats is evident from the results presented.

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Effects of Plasma Erythropoiesis Stimulating Factor (E.S.F.) at Different Time Intervals After Single Injection. (26469)

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It has been suggested(1,2) that erythropoiesis stimulating factor (E.S.F) stimulates differentiation of stem cells into red cell precursors. Results of histological study of the spleen of polycythemic mice treated with E.S.F. support this view(3). More detailed analysis of several effects of E.S.F. might provide evidence for or against this theory. Therefore, amino acid and iron incorporation in hemoglobin, and reticulocyte levels were measured at various time intervals after a single intravenous injection of E.S.F.

Methods. Normal Webster Swiss female mice 24-30 g obtained from Diablo, Berkeley, Cal. were used. E.S.F. extract, No. LD11, was prepared from plasma of rabbits made severely anemic with phenylhydrazine by methods already described(4). Its activity was about 0.7 CS 1 unit/mg or 7 Co units/mg. This extract is of the same type as Extract CS 1 which was assayed in several laboratories(4).

Several criteria were employed to measure the effects of the extract. a) Measurement of C^{14} amino acid uptake in globin as proposed by Dovey *et al.*(5). b) Measurement of Fe^{59} in plasma and spleen at 2 hours and in red cells at 48 hours after tracer injection. c) Reticulocyte counts. d) Measurement of reticulocytes by determining *in vitro* uptake of Fe^{59} and C^{14} leucine into washed red cells from 5 mice, by methods described by Borsook(6).

The experimental design was the following: on day 0 the mice received 0.25 ml of .9% NaCl solution containing 2 mg LD11 intravenously (tail vein). Controls received .9% NaCl solution. For the C^{14} amino acid experiments groups of mice—number of animals shown in Table I—were injected intravenously at 12, 24, 48, 72, and 96 hours after the extract with 1 μc C^{14} uniformly labelled amino acids obtained by acid hydrolysis of C^{14} labelled chlorella protein (CFB6 Radiochemical center, Amersham, England) with a specific activity of 1 mc/5.6 mg. One week later, a time when C^{14} activity globin had levelled off, mice were exsanguinated by heart puncture under ether anesthesia. In initial experiments pooled red cells from 6 mice were washed thrice with 30 vol. of ice cold .9% NaCl; 0.10 ml of the red cell suspension was dried on planchettes using the technic described by Lowy *et al.*(7), and counted in Nuclear Chicago automatic gas flow counter. The rest of the red cells were lysed with 20 vol. of distilled water, insoluble material was centrifuged down, the clear super-

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