

The Influence of 1-Amino-1-Cyclopentane Carboxylic Acid (Cycloleucine) on Food Efficiency in Rats (40691)¹

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Cycloleucine, chemically known as 1-aminocyclopentane-1-carboxylic acid (ACPC) is not a naturally occurring amino acid (see Figure 1). Metabolic studies of ACPC in mice and rats have shown that it is not metabolized nor excreted rapidly; therefore, blood and peripheral tissue levels are maintained for several days (1, 2). Ross et al (3) reported a decreased growth rate in animals fed a diet containing ACPC and suggested it may function as an amino acid antagonist. Clark et al (4) proposed that its effect on growth rate and amino acid pools were secondary to a depressed food intake.

The results obtained in the present study suggest yet another mechanism of action of ACPC on reducing food efficiency in rats fed a purified high starch diet.

Materials and methods. Male Wistar rats (160–170 g) were given a standard laboratory Purina Chow or high carbohydrate diet² in powdered form *ad libitum* to facilitate estimation of daily food consumption. They were housed individually in open wire bottom cages. Water was available at all times. In experiment I, 5 days after commencement of the high carbohydrate diet, groups of animals received either the basal ration or ACPC incorporated into the diet at the following levels: 0.0125, 0.025, 0.05, or 0.1%. In experiment II, ACPC and its chemically related amino acids (L-(–)-proline, L-4-hydroxyproline, glycine, L-leucine, or L-isoleucine) were admixed separately to the Purina Chow diet at 0.5 mg/g diet (0.05%) or 1 mg/g diet (0.1%), respectively. Animals were given *ad libitum* for a period of 3 days.

¹ Part of this work was presented at the First Biochemistry Section Meeting of the New Jersey Academy of Science at Camden, N.J. in April 1976.

² High-carbohydrate diet, obtained from Nutritional Biochemical Co., Cleveland, Ohio with the following composition: "vitamin-free" casein 18%; starch 68%; vegetable oil 8%; brewer yeast U.S.P. 2%; salt mixture U.S.P. No. 2, 4%; plus ICN vitamin diet fortification mixture.

Body weight and food consumption were monitored throughout experimental period. Blood was obtained by cardiac puncture and portions of the pancreas, small intestine, and liver were collected and chilled rapidly from anesthetized rats at the end of the studies.

Serum sugar and insulin determinations. Serum levels of reducing sugars were measured with a Technicon Auto Analyzer (Technicon Method N-2b) whereas insulin levels were estimated by radioimmunoassay (New England Nuclear).

Determination of amylase activity. Pancreas and liver sections were weighed and homogenized in cold 0.9% sodium chloride. Whole homogenates were used for α -amylase estimation. A microadaptation of the starch-iodine method of Street (5) was used for amylase activity determination. A 1% soluble starch was dissolved in 20 mM phosphate buffer at pH 6.9 containing 6.9 mM NaCl by heating. Test tubes containing the diluted enzyme solution and buffer to a final volume of 0.4 ml were placed in a water bath (37°) for 3–5 min. A sufficient amount of the starch solution was at the same time placed in another test tube in the water bath.

Subsequently 0.1 ml of the starch solution was transferred to the tubes containing the diluted enzyme. The solutions were mixed and at the same time a stopwatch was started. After exactly 3 min of incubation, the absorbance of the starch-iodine complex obtained on addition of 0.1 ml digest to 5.0 ml of 0.02% iodine–0.2% KI solution was measured in a Bausch & Lomb Spectronic 20 at 680 nm. The unit of amylase activity is expressed as the amount of enzyme sufficient to hydrolyze 1 mg of starch in 3 min at 37°.

Assay of disaccharidase activity. A 15-cm segment of small intestine (the first portion of 5-cm segment was discarded) was separated and flushed with 3 to 5 ml of cold distilled water.

This segment was cut open and laid on a

cold sheet of glass placed on crushed ice. The mucosa was scraped off with a microscopic glass slide and homogenized with 4.0 ml cold distilled water. The test tubes were chilled with crushed ice before and during homogenization. Nuclei and larger cell debris were removed by centrifugation at 500 g for 20 min at 4°.

The supernatant contained the disaccharidases. Maltase, sucrase, and lactase activities were assayed by the Tris-glucose oxidase method as described by Dahlqvist (6). One unit of disaccharidase activity is expressed as micromoles of substrate hydrolyzed per minute.

Pancreatic lipase activity. Lipase was prepared by extracting rat pancreas ethanol-acetone powder with water. The supernatant solution obtained upon centrifugation was used to assay for lipase activity according to Marchis-Mouren *et al.* (7) by the use of a pH-stat (Radiometer titrigraph). Protein was de-

termined according to Lowry *et al.* (8), using Folin-Ciocalteu reagent. The specific activity of a lipase solution is defined as the number of lipase units/mg protein.

Results and discussion. Control rats in experiment I gained an average of 28 g during the experimental period. Animals receiving ACPC in their diet showed a dose-related loss in body weight gain (Table I) associated with reduced food efficiency (Fig. 2). Food consumption, however, was not significantly reduced except at the highest (0.1%) concentration of ACPC in the diet. Levels of serum sugar, insulin, and amylase activity were not altered by ACPC treatment (Table II).

Pancreatic α -amylase activity was markedly reduced at all levels of ACPC treatment but was statistically significant at the two highest concentrations only (Table III). Conversely, hepatic α -amylase activity increased in a dose-related manner being significant at the three highest concentrations of ACPC in the diet (Table III). The overall hepatic α -amylase activity was found to be relatively small compared with that of the pancreas.

Table IV shows the intestinal disaccharidase activities in rats fed a high carbohydrate diet containing 0.1% ACPC. The activities of all three hydrolases (maltase, sucrase, and

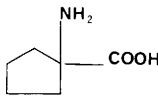


FIG. 1. The chemical structure of cycloleucine—ACPC.

TABLE I. EFFECT OF ACPC ON WEIGHT GAIN AND FOOD CONSUMPTION IN RATS (3 DAYS ON PURIFIED DIET)

% of ACPC in diet	Number of rats	Weight gain—3 days		Food consumption—3 days	
		g (mean \pm SEM)	Change as % of control	g (mean \pm SEM)	Change as % of control
0	5	28.0 \pm 1.7	—	63.6 \pm 2.9	—
0.0125	5	22.4 \pm 2.4	\downarrow 20	56.2 \pm 3.7	\downarrow 12
0.025	6	20.7 \pm 2.1	\downarrow 26*	60.0 \pm 2.8	\downarrow 6
0.05	6	19.7 \pm 4.3	\downarrow 30*	60.2 \pm 5.3	\downarrow 5
0.1	5	10.2 \pm 1.7	\downarrow 64**	48.3 \pm 2.5	\downarrow 24**

* Statistically significant from control, $P \approx 0.01$.

** Statistically significant from control, $P < 0.001$.

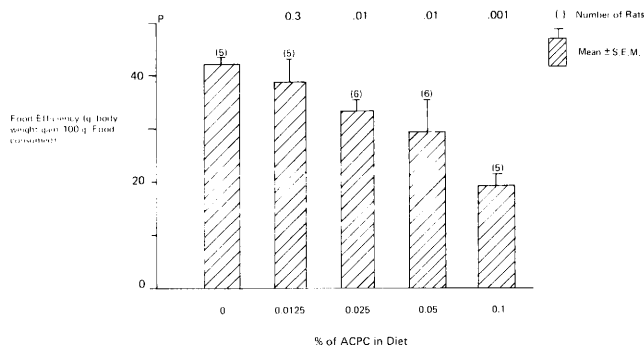


FIG. 2. The effect of ACPC on food efficiency in rats.

TABLE II. EFFECT OF ACPC ON SERUM SUGARS, INSULIN, AND α -AMYLASE

% of ACPC in diet	Number of rats	Serum sugars mg% (mean \pm SEM)	Serum insulin μ units/ml (mean \pm SEM)	Serum α -amylase activity units/ml (mean \pm SEM)
0	5	167 \pm 5	50 \pm 2	20.42 \pm 0.64
0.0125	5	172 \pm 7	50 \pm 17	20.20 \pm 1.50
0.025	6	163 \pm 3	52 \pm 7	19.79 \pm 0.89
0.05	6	168 \pm 4	55 \pm 8	19.44 \pm 0.62
0.1	5	165 \pm 2	45 \pm 5	18.89 \pm 1.13

lactase) were not altered by ACPC treatments. However, pancreatic lipase was reduced to about a third of that observed in the absence of ACPC but was not statistically significant from the control.

Results obtained in experiment II are presented in Table V. A decrease in food efficiency was not observed when other amino acids were incorporated into the diet. These results are in contrast to those obtained when ACPC was incorporated into the diet.

TABLE III. EFFECT OF ACPC ON PANCREATIC AND HEPATIC α -AMYLASE ACTIVITIES

% of ACPC in diet	Number of rats	Pancreatic α -amylase activity		Hepatic α -amylase activity	
		units/100 mg tissue (mean \pm SEM)	Change as % of control	units/100 mg tissue (mean \pm SEM)	Change as % of control
0	5	588 \pm 76	—	0.54 \pm 0.05	—
0.0125	5	398 \pm 90	\downarrow 32	0.69 \pm 0.14	\uparrow 28
0.025	5	518 \pm 71	\downarrow 12	1.47 \pm 0.21	\uparrow 172**
0.05	6	336 \pm 27	\downarrow 43*	1.59 \pm 0.17	\uparrow 194**
0.1	5	241 \pm 21	\downarrow 59**	3.64 \pm 0.74	\uparrow 574**

* Statistically significant from control, $P \approx 0.01$.

** Statistically significant from control, $P < 0.001$.

TABLE IV. EFFECT OF ACPC ON RAT INTESTINAL BORDER DISACCHARIDASES AND PANCREATIC LIPASE

Treatment	Disaccharidases ^a						Lipase ^b	
	Maltase		Sucrase		Lactase		units/mg protein (mean \pm SEM)	Change as % of control
	units/g protein (mean \pm SEM)	Change as % of control	units/g protein (mean \pm SEM)	Change as % of control	units/g protein (mean \pm SEM)	Change as % of control		
Control	275 \pm 23	—	61 \pm 5	—	7.8 \pm 1.5	—	3.2 \pm 0.62	—
ACPC 0.1%	227 \pm 16	\downarrow 17	73 \pm 5	\uparrow 20	7.1 \pm 1.1	\downarrow 9	2.2 \pm 0.50	\downarrow 31

^a Each treatment consists of six rats.

^b Each treatment consists of five rats.

TABLE V. EFFECT OF DIFFERENT AMINO ACIDS ON BODY WEIGHT GAIN, FOOD CONSUMPTION, AND FOOD EFFICIENCY (3 DAYS ON PURINA CHOW)

Amino acids	% in diet ^a	Body weight gain—3 days		Food consumption—3 days		Food efficiency	
		Grams (mean \pm SEM)	Change as % of control	Grams (mean \pm SEM)	Change as % of control	Grams body weight gain/100 g food (mean \pm SEM)	Change as % of control
None	—	28 \pm 2	—	76 \pm 3	—	37 \pm 3	—
Cycloleucine (ACPC)	0.05	13 \pm 3	\downarrow 54***	62 \pm 5	\downarrow 18*	21 \pm 5	\downarrow 43**
L-(—)-Proline	0.10	26 \pm 2	\downarrow 7	71 \pm 3	\downarrow 7	36 \pm 2	\downarrow 3
L-4-Hydroxyproline	0.10	27 \pm 3	\downarrow 4	76 \pm 2	0	36 \pm 4	\downarrow 3
Glycine	0.10	27 \pm 1	\downarrow 4	79 \pm 3	\uparrow 4	40 \pm 7	\downarrow 8
L-Leucine	0.10	25 \pm 2	\downarrow 11	73 \pm 2	\downarrow 4	35 \pm 2	\downarrow 5
L-Isoleucine	0.10	29 \pm 2	\uparrow 4	75 \pm 2	\downarrow 1	35 \pm 3	\downarrow 5

^a Each treatment consists of six rats.

* Statistically significant from control, $P = 0.05$.

** Statistically significant from control, $P \approx 0.01$.

*** Statistically significant from control, $P < 0.001$.

Clark and his co-workers (4) showed that the decreased growth rate of ACPC treated rats was secondary to the effect on reduced food intake when animals were on a high glucose (65.8%) diet. In other words, reduction of body weight gain was seen only when the food consumption was affected. In our experiment, decrease in body weight gain of ACPC-treated rats (0.0125–0.05% in the diet) was not related to reduced food intake. However, 0.1% ACPC caused a marked decrease in body weight and a weak to moderate effect on food consumption.

The activities of other digestive enzymes such as intestinal maltase, sucrase, lactase, and pancreatic lipase were not significantly affected by ACPC treatment. However, production of pancreatic α -amylase is essential for converting starch into maltose which is broken down into glucose, the predominant form of carbohydrate prior to being absorbed in the gastrointestinal tract. Retardation or inhibition of pancreatic α -amylase by ACPC would reduce food efficiency by limiting starch digestion thereby reducing available glucose for absorption causing caloric imbalance resulting in the loss of body weight. In the study reported by Clark *et al.* (4), glucose was present in the diet as the carbohydrate source, and α -amylase would have no influence on the glucose absorption. Therefore, the reduction of body weight gain in rat maintained on glucose-rich diet was probably due to the lowering of food intake. In the current study the loss of body weight and lowering of food efficiency may be attributed, in part, to the reduction of pancreatic α -amylase caused by ACPC.

It was also interesting to note that the serum insulin level was not affected by ACPC treatment, an indication that at least the β -cells of the pancreas were not effected in rats after 3 days of ACPC treatment. The increase of α -amylase activity in the livers of ACPC-treated rats was an unexpected response. At this time, we do not have any explanation for this observation.

Chemically related amino acids, proline, hydroxyproline, glycine, leucine, and isoleucine did not show any influence on food efficiency in rats. The lowering of pancreatic α -amylase activity in ACPC-treated rat is considered the specific cause of the decrease of food efficiency in this study.

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