

altered by exposure of the mice to oxygen at 45 p.s.i.a.

Ross and McAllister(3) concluded that oxygen therapy significantly prolonged life in 2 out of 8 experiments with mice injected with *D. pneumoniae* intraperitoneally. It is difficult to evaluate protection in their experiments; with the inoculum used, 90% of mice were usually dead within 24 hours after infection. In the present study following intravenous infection with much smaller inocula and delayed death, no protection was observed in mice treated with hyperbaric oxygen.

Grogan(7) found that oxygen at 45 p.s.i.a. did not protect mice from death following infection with *S. aureus* intraperitoneally. In fact, he concluded that oxygen therapy increased mortality. In his studies exposures to oxygen lasted 2 to 3 hours which in the present study caused deaths in normal mice. Although in the present study no protection was afforded by oxygen therapy of aerobic infections, it is also of note that no increase in mortality from infection occurred in mice exposed to oxygen.

Summary. Strains of *E. coli*, *A. aerogenes*, *P. aeruginosa*, *S. typhimurium*, *P. mirabilis*, *D. pneumoniae*, *S. aureus*, and *Strep. pyogenes* in broth multiply in oxygen at 30 pounds per square inch absolute (p.s.i.a.). The rate of growth and the peak titer achieved were frequently inhibited in oxygen as compared with air at 15 p.s.i.a. The inhibitory

effect of oxygen was most marked with *S. typhimurium*, *D. pneumoniae* and *S. aureus* and was minimal or absent with *P. mirabilis*, *A. aerogenes* and *E. coli*. In general, 24-hour cultures were more susceptible to the inhibitory activity of oxygen than were 4-hour cultures. Each of the strains of aerobic bacteria grew on the surfaces of agar plates when incubated in oxygen or air. Although equal numbers of colonies developed in oxygen and air, differences in colonial morphology were noted in some of the strains incubated in oxygen. Exposure to oxygen at 45 p.s.i.a. did not protect mice against death from infection produced by intravenous administration of *D. pneumoniae*, *S. aureus*, or *S. typhimurium*.

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Food Intake, Weight Gain and Amino Acid Pools of Rats Receiving 1-Aminocyclopentane-1-Carboxylic Acid.* (31933)

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Christensen and Jones(1) observed that the cyclic amino acid, 1-aminocyclopentane-1-carboxylic acid (ACPC) when injected into the rat is not metabolized and maintains a steady-state concentration in the blood and tissues

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for several days. These biological characteristics led Akedo and Christensen(2) to propose the use of ACPC as a model for the study of amino acid transport. In experiments with rats fed diets containing ACPC, Ross *et al*(3) reported a decreased growth rate and suggested that the compound might function as an amino acid antagonist. Berlinguet *et al*

(4) obtained evidence indicating that ACPC inhibited protein synthesis *in vivo* and *in vitro* by preventing the attachment of valine to transfer RNA. Thus, it seemed possible that *in vivo*, the presence of ACPC might alter the distribution of amino acids in the tissue pools and a study was designed to measure the levels of amino acids in plasma, liver and muscle of rats receiving ACPC.

Materials and methods. Male rats (210 g) of the Sprague-Dawley strain were randomly allotted to the treatments in each experiment. The basal diet (%) consisted of the following: casein, 18.0; glucose, 65.8; corn oil, 10.0; mineral mixture(5), 4.0, and vitamin mixture,† 2.2. The vitamin mixture has been described previously(6). All animals were allowed the basal diet *ad libitum* for several days prior to initiation of the experiments.

In Experiment I, one group of rats received the basal diet (control) and a second group the basal diet supplemented with 0.1% ACPC (substituted for an equal amount of glucose). The animals were allowed the diets *ad libitum* for a period of 3 days.

Since it was found that animals receiving ACPC had a reduced food intake, in Experiments II and III, feeding was controlled by utilizing a plastic stomach tube. For the tube feeding, the basal diet was mixed with tap water, homogenized in a Waring blender and diluted to a total volume in ml that was equivalent to the g of dry diet. The animals were fed by stomach tube for 2 days prior to initiation of the experiments to accustom them to the feeding procedure. In a preliminary experiment, the growth rate and food intake of rats injected intraperitoneally with 60 mg of ACPC were found to be similar to the data reported in Experiment I. Therefore the rats in Experiments II and III were injected intraperitoneally with 60 mg of ACPC while the control animals received a saline solution.

In Experiment II, the rats received 6.2 g of the diet 3 times daily for 3 days. In Experiment III, the rats received 5 g of diet 4 times per day and the experiment was continued for 1 week.

†The vitamin mixture was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

At termination of the experiments, the rats were anesthetized with ether, and blood (cardiac puncture) and tissues were removed for subsequent amino acid analysis. Amino acid analyses were performed by ion exchange chromatography(7) on deproteinized supernatants of plasma, liver and muscle (gastrocnemius). The methods used have been described previously(6). During the development of the chromatogram, it was observed that ACPC was eluted between the leucine and tyrosine peaks and this amino acid could therefore be quantitated. The basic amino acids were not measured.

Results and discussion. In Experiment I, the rats receiving the basal diet (Table I) showed a total average body weight gain of 32.2 g whereas the animals receiving the diet containing 0.1% ACPC were unable to maintain their initial body weight. The average food intake was also greatly decreased for rats ingesting the diet containing ACPC as compared to the rats fed the basal diet.

Many of the essential and nonessential amino acids which were analyzed in the amino acid pools of rats receiving ACPC were significantly decreased in concentration (Table I) as compared to values obtained in rats given the basal diet. Berlinguet *et al*(4) found that ACPC prevented the *in vivo* and *in vitro* incorporation of valine into rat proteins, and this finding might be expected to result in increased valine levels in plasma. However, in this experiment, valine was one of a number of amino acids that were markedly reduced in concentration in the amino acid pools. Fewer alterations occurred in the amino acid levels in liver and muscle of rats given ACPC and the changes for glycine and serine in these tissues represented increases over values found in rats receiving the basal diet.

In Experiment II, where food intake was kept constant by stomach tube feeding, concentrations of ACPC in the amino acid pools (Table II) from animals injected with ACPC were considerably higher than the levels of ACPC in tissues from rats in Experiment I; therefore, it would appear that the injected amount of ACPC was greater than the amount the animals ingested with the diet containing

ACPC. However, the value of ACPC in the plasma of rats (Experiment III, Table II) found 7 days after injection was only slightly higher than the value of ACPC in rats in Experiment I.

In contrast to the first experiment, the rats receiving ACPC gained weight in Experiment II, an average of 4.4 g (Table II) for 3 days as compared to 7.5 g for the rats injected with saline. The growth rate of the rats on both

TABLE I. Effect of ACPC on Body Weight Gain and Amino Acid Pools* of Rats Allowed Diets *ad libitum*.

Amino acid	Plasma		Liver		Muscle	
	Control diet	ACPC diet	Control diet	ACPC diet	Control diet	ACPC diet
Thr	58.1 ± 3.0	39.6 ± 4.3§	80.5 ± 2.3	67.5 ± 6.1	156.6 ± 27.0	143.3 ± 4.8
Val	27.1 ± 1.2	14.6 ± 4.3§	32.5 ± 1.4	15.3 ± 7.4‡	29.9 ± 4.5	21.3 ± 2.2
Met	6.8 ± .9	4.8 ± .0‡	10.9 ± 6.3	9.3 ± 3.6	6.8 ± 3.3	4.1 ± 1.0
Ileu	12.4 ± .8	7.2 ± 1.3§	20.8 ± 1.4	17.2 ± 1.6‡	13.3 ± 2.6	13.5 ± 1.7
Leu	19.2 ± 1.2	10.4 ± 2.0§	33.3 ± 1.6	29.9 ± 1.6	20.7 ± 3.5	18.1 ± 2.2
Tyr	16.6 ± .9	7.9 ± 1.1§	12.1 ± 1.7	10.1 ± 1.2	25.3 ± 4.5	15.5 ± .5
Phe	7.2 ± .9	6.4 ± .9	10.4 ± 3.3	10.9 ± 2.7	10.4 ± 1.9	11.8 ± .8
Ser	33.2 ± 1.8	37.2 ± 6.9	103.0 ± 9.0	117.2 ± 17.8	139.2 ± 7.0	188.0 ± 12.3§
Asp-Glu	81.6 ± 2.5	64.0 ± 10.4‡	806.9 ± 90.6	867.3 ± 55.3	446.2 ± 27.8	404.3 ± 96.9
Pro	40.0 ± 6.6	18.9 ± 2.2§	—	—	94.8 ± 27.9	47.9 ± 2.6§
Glu acid	11.8 ± 1.5	11.9 ± 2.0	297.3 ± 3.7	192.6 ± 28.8§	117.0 ± 5.2	88.7 ± 12.3‡
Gly	20.0 ± 1.4	26.0 ± 3.1‡	208.4 ± 15.4	255.7 ± 21.3‡	231.9 ± 51.5	361.8 ± 25.7‡
Ala	58.5 ± 4.0	34.8 ± 1.3§	446.9 ± 36.0	336.5 ± 51.6‡	391.8 ± 72.1	310.6 ± 17.5
ACPC	—	127.6 ± 9.9	—	171.3 ± 18.8	—	162.9 ± 18.5
Total mean body wt gain (g)†	+32.2 ± 4.4	-2.4 ± 3.1§				
Total mean food intake/rat (g)	75.0 ± 6.7	40.8 ± 6.4§				

* Each value is the mean ± S.D. of 3 determinations of pooled samples from 4 non-fasting rats in μmoles/100 ml for plasma and in μmoles/100 g wet weight for liver and muscle.

† There were 12 rats per treatment.

‡ P < .05; § P < .01; significant difference from control values by t test.

TABLE II. Effect of ACPC on Body Weight Gain and Amino Acid Pools of Rats Fed by Stomach Tube.

Amino acid	Experiment II				Experiment III	
	Plasma*		Liver†		Plasma‡	
	Saline	ACPC	Saline	ACPC	Saline	ACPC
Thr	29.0 ± 5.6	32.4 ± 5.4	35.7 ± 2.8	38.2 ± 11.2	34.8 ± 2.2	32.0 ± 3.9
Val	16.1 ± 2.1	16.2 ± 2.2	23.5 ± 6.6	29.0 ± 1.8	15.9 ± .2	17.5 ± 1.3
Met	3.9 ± .4	5.2 ± .6	3.0 ± 1.3	5.2 ± .4§	3.8 ± .5	3.9 ± .3
Ileu	7.8 ± 1.5	7.1 ± .9	16.3 ± 4.0	16.6 ± 3.3	7.8 ± .4	7.9 ± .8
Leu	12.2 ± 1.4	11.6 ± 1.9	28.2 ± 4.6	29.0 ± 3.4	12.7 ± .3	12.8 ± 1.3
Tyr	8.2 ± .9	9.0 ± 1.5	11.3 ± .8	10.8 ± 1.5	9.0 ± .4	7.9 ± .3
Asp-Glu	58.9 ± 4.8	59.2 ± 6.1	744.5 ± 182.8	749.0 ± 95.6	41.5 ± 1.5	34.0 ± 4.0
Pro	16.1 ± 3.7	21.8 ± 5.2§			13.2 ± 1.2	21.8 ± 1.9
Glu acid	11.5 ± 2.6	9.0 ± 1.5	392.0 ± 112.9	269.0 ± 101.9	22.3 ± 1.7	19.3 ± 2.4
Gly	22.4 ± 1.5	19.0 ± 4.8	282.0 ± 18.4	211.0 ± 20.1	19.0 ± 1.5	13.6 ± 1.5§
Ala	52.3 ± 12.7	44.3 ± 7.1	375.0 ± 68.2	418.0 ± 133.5	46.5 ± 1.6	40.8 ± 4.2
ACPC		271.8 ± 16.7		308.0 ± 93.2		154.7 ± 10.1
Total mean body wt gain (g)	7.5 ± 3.8	4.0 ± 3.7			30.9 ± 6.9	27.2 ± 7.1
No. of animals	6	8			9	9

* Each value is the mean ± S.D. in μmoles/100 ml from individual determinations. The rats were sacrificed at 13 hr after the last feeding.

† Each value for liver is the mean ± S.D. in μmoles/100 g wet weight from 3 and 4 determinations from pooled samples of 2 rats for treatments 1 and 2, respectively.

‡ Each value is the mean ± S.D. in μmoles/100 ml from 3 determinations from pooled samples of 3 rats. The rats were sacrificed at 13 hr after the last feeding.

§ P < .05; || P < .01; significant difference from control values by t test.

treatments was slow as compared to the expected growth rate of rats given this type of diet and could perhaps be attributed to the feeding procedure. As a result of the modified feeding procedure in Experiment III, the total body weight gains of the rats were greatly increased (Table II). The rats injected with saline averaged 30.9 g for 7 days and those injected with ACPC averaged 27.2 g. No significant difference was observed in the growth rate among the animals injected with saline and those injected with ACPC in either experiment.

In Table II are shown the values obtained in Experiments II and III of amino acids that were significantly altered in plasma and tissues in Experiment I. In neither Experiment II nor III were there significant decreases in any of the amino acids in rats receiving ACPC. The amino acid values for the muscle (data not tabulated) likewise showed no decreases. Some amino acids such as methionine and proline appeared to be elevated in concentrations in rats given ACPC. These results indicate that the amino acid levels in plasma and tissues were not decreased by ACPC administration when food intake was maintained. They further suggest that the effects of ACPC on growth rate and the distribution of amino acids in amino acid pools of animals allowed food *ad libitum* are secondary to a depressed food intake.

Machlin *et al*(8) reported that valine supplementation alleviated the growth retardation of chicks fed diets containing ACPC and we have also observed that 1% valine added to the diet prevented decreased growth rates and alterations in amino acid pools of rats (results not tabulated) fed diets containing ACPC. It would appear both from these results and those of Berlinguet *et al*(4) that ACPC functions as a valine antagonist. However, from the observations with controlled food intake as reported here, it would also appear that a sufficient amount of valine is

present in the basal diet to prevent the adverse effects of ACPC if the diet is consumed in adequate amounts.

The results of Berlinguet *et al*(4) show that ACPC prevents the formation of the valine-sRNA complex. Since the present experiments have indicated that the biological effects of ACPC are secondary to a decrease in food intake, it would appear that the aminoacyl-sRNA complex should be considered as a necessary component of some appetite regulatory mechanism.

Summary. Rats allowed diets containing 0.1% ACPC *ad libitum* for a 3-day period exhibited decreases in food intake and growth rate as compared to animals given the basal diet. Significant reductions occurred in the levels of the amino acids in plasma and tissues of rats receiving ACPC. When food intake was maintained by feeding with a stomach tube, no appreciable differences were found in growth rate or in the composition of the amino acid pools of animals injected with ACPC as compared to animals injected with saline. It would appear that ACPC does not affect the distribution of amino acids in animal tissues or the growth rate when food intake is controlled.

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