days of extract injection. The reduction of acetonuria associated with succinate and glucose administration is indicated. This diminution is statistically significant except for the 3rd day of succinate. The fact that glucose is more efficient and the fact that succinic acid may be converted to glucose in the body[3] suggest that sodium succinate may exert its effect because of its conversion to glucose.

TABLE I.

Series	No. of Rats	Avg wt	Wt loss, avg	Avg acetone body excretion in mg per 100 g body wt† day of fast		
				1	2*	3*
A	17	185.1	23.8	1.24	21.28 ± 1.80	23.41 ± 2.21
B	17	183.6	26.4	0.94	15.89 ± 0.93	20.95 ± 0.84
C	17	189.2	21.3	1.01	8.74 ± 0.47	10.46 ± 0.52

A = Fasting.
B = Fasting with Succinate
C = Fasting with Glucose.
* Given anterior pituitary extract 0.5 cc per 100 g body weight.
† With standard error of mean.

These results are comparable to the effects of succinic acid and glucose on the ketonuria produced by high fat diets.[1] It appears that experimental ketonuria produced in 2 ways, by high fat diets or by fasting and anterior pituitary extract, may be diminished by succinic acid, but that this substance acts much less effectively than glucose.

10182

Availability of Dibenzoylcystine for Growth of the Young White Rat.

PONG C. JEN AND HOWARD B. LEWIS.

From the Department of Biological Chemistry, University of Michigan, Ann Arbor.

If the α-amino group of cystine was blocked by replacement of a hydrogen atom with a benzoyl group, the sulfur of the resulting dibenzoylcystine was not oxidized readily to sulfate sulfur in the organism of the rabbit, but a large part of the compound was excreted by the kidneys, either unchanged or as the corresponding cysteine derivative.[1] Since the biological oxidation of this α-substi-

[3] Ringer, A. I., Frankel, E. M., and Jonas, L., *J. Biol. Chem.*, 1913, **14**, 539.

[1] Lewis, H. B., Updegraff, H., and McGinty, D. A., *J. Biol. Chem.*, 1924, **59**, 59.

tuted cystine derivative to sulfate was almost negligible after its parenteral administration, while the oxidation of the sulfur of dibenzoylcystine after oral administration, although variable and not marked, was considered significant, it was suggested that orally administered dibenzoylcystine was hydrolyzed in part to yield cystine and benzoic acid, prior to absorption from the alimentary tract, by microbial or other agencies.

A second method of attack on this problem is the study of the supplementary effect of dibenzoylcystine in the diet on the growth of young white rats fed a diet known to be deficient in its content of sulfur-containing amino acids. The utilization for purposes of growth of a cystine derivative is usually assumed to be evidence of the conversion of the derivative to cystine in the body, although this interpretation is not necessarily the only possible one. Jones, Andrews and Andrews[2] reported that rats fed dibenzoylcystine as a supplement to a basal diet low in its cystine content showed occasional irregular increases in the rate of growth in comparison with control animals receiving the basal diet. This suggested that hydrolysis of dibenzoylcystine might proceed under certain conditions with sufficient rapidity to permit this cystine derivative to serve as a source of cystine for purposes of growth of the young white rat.

Since any significant biological hydrolysis of dibenzoylcystine would result in the liberation of benzoic acid, which may influence unfavorably the growth of rats, especially when diets low in protein (casein) are fed,[3] it seemed that optimal conditions for the observation of any growth-promoting effect of dibenzoylcystine might be obtained, if glycine, a non-essential amino acid, were also added to aid in the detoxication of any benzoic acid produced by the hydrolysis of the dibenzoylcystine. The presence or absence of glycine in the diet should not influence the rate of growth, if benzoic acid were not a dietary component[3] as such or formed in the metabolic processes.

Young white rats in litter units were fed a basal diet low in its content of cystine[4] for a preliminary feeding period of 14 days. The animals either failed to gain or showed almost negligible increases in weight. Water-soluble and fat-soluble vitamins were

2 Jones, J. H., Andrews, K. C., and Andrews, J. C., *J. Biol. Chem.*, 1935, **109**, xlviii.

3 Griffith, W. H., *J. Biol. Chem.*, 1929, **82**, 415.

4 White, A., *J. Biol. Chem.*, 1936, **112**, 503. The diet was altered to contain 5% instead of 6% of casein (Labco Brand, vitamin-free) and 1% of agar.

supplied by the separate administration of dried brewer's yeast (approximately 400 mg) and cod liver oil (3 drops) daily. After the preliminary feeding period, the animals were divided into 3 groups and the paired feeding method employed. One group received supplementary cystine (0.5%); a second group received dibenzoyl-*l*-cystine[5] in an amount equivalent in sulfur content to 0.5% of cystine; and the third group was continued on the basal diet. Glycine (0.5%) was added to the diets of all 3 groups. The experimental period included 50 days.

TABLE I.

Food Consumption and Increases of Weight of Rats Fed a Basal (Cystine Deficient) Diet Supplemented by Cystine and Dibenzoylcystine Over a Period of 50 Days.

Glycine was supplied in all the diets. All values are expressed as grams. The letters after the numbers of the animals indicate sex. The pairings for the controlled feedings were as follows: Rats 4211, 4413 and 4119; 4225, 4424 and 4127; 4238 and 4436.

Supplement	Cystine			Dibenzoylcystine			None	
Rat No.	4211M	4225F	4238F	4413M	4424F	4436F	4119M	4127F
Initial wt	51	53	54	59	59	61	75	50
Final wt	117	116	117	79	75	66	96	66
Total gain	66	63	63	20	16	5	21	16
Avg daily gain	1.32	1.26	1.26	0.40	0.32	0.10	0.42	0.32
Total food	275	278	289	282	286	297	278	283
Avg daily food	5.5	5.6	5.8	5.6	5.7	5.9	5.6	5.7
Gain per 100 g of food	24.0	22.6	21.8	7.1	5.6	1.7	7.5	5.6

The results with one litter are presented in Table I and require little comment. The animals receiving cystine and glycine as supplements gained weight rapidly, an average weight increment of 22.8 g per 100 g of food consumed, while the animals which received dibenzoylcystine and glycine showed no better growth than the animals fed the basal diet supplemented with glycine. It is evident that under our experimental conditions, even though dietary glycine were available to detoxicate any benzoic acid which might be formed in the biological hydrolysis of the benzoyl derivative of cystine, this hydrolysis did not *proceed with sufficient rapidity* to permit dibenzoylcystine to serve as an *effective* source of cystine for the purposes of growth of the young white rat. The hydrolysis of dibenzoylcystine, if it occurs in the gastro-intestinal tract of the young white rat, is probably without any biological significance.

Summary. Young white rats were fed *l*-cystine and dibenzoyl-*l*-cystine as supplements to a basal diet known to be low in its content

[5] Curtius, T., and Kyriacou, N. C., *J. prakt. Chem.*, 1917, **95**, 360.

of cystine, in an attempt to determine whether dibenzoyl-*l*-cystine could be hydrolyzed in the organism and thus serve as a significant source of cystine for purposes of growth. To detoxicate any benzoic acid which might be formed in the hydrolysis and afford optimal conditions for observation of the growth-promoting effect of any cystine formed, glycine was also added to the diets. No evidence was obtained that dibenzoyl-*l*-cystine under the experimental conditions employed could serve as an effective source of cystine for purposes of growth.

10183

Effect of Methionine on Casein Metabolism.*

M. C. KIK. (Introduced by Barnett Sure.)

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark.

Many investigators have reported that cystine stimulates growth in rats fed a ration containing casein as the sole protein. This ration is low in cystine. Mitchell,[1] Greaves and Morgan,[2] and Kik[3] have also shown that the biological value of casein, as determined by short metabolism experiments according to Mitchell's method, increased after addition of cystine.

Some years ago, Jackson and Block[4] produced evidence that methionine like cystine is capable of stimulating growth in rats fed a low cystine diet and quite recently Rose and coworkers[5] proved that methionine is an indispensable amino acid, 0.6% of which is needed in the diet for a normal increase in weight. According to them cystine is dispensable and stimulates growth only when methionine is present in too low a level.

In the present communication results are reported of a metabolism experiment with rats for determining the effect of methionine versus cystine addition on the biological value of casein for maintenance and growth, as evidenced by nitrogen balance experiments.

* Research paper No. 481, Journal Series, University of Arkansas.

1 Mitchell, H. H., *J. Biol. Chem.*, 1924, **58**, 923.

2 Greaves, E. D., and Morgan, A. F., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 506.

3 Kik, M. C., *Ark. Agr. Exp. Sta. Bull.* 352, 1938.

4 Jackson, R. W., and Block, R. J., *J. Biol. Chem.*, 1932, **98**, 465.

5 Womack, M., Kemmerer, K. S., and Rose, W. C., *J. Biol. Chem.*, 1937, **121**, 403.