in general had a wide range of M_r's (between 14 000 and 60 000) and pI's. Since, in the NEPHGE system the pH gradient is not allowed to reach equilibrium to prevent cathodic pH drift (O'Farrell et al., 1977; Horst et al., 1980), it will not be possible to obtain accurate pI's of the polypeptides. Hence, pI's of the polypeptides were not determined.

These data indicated that the basic proteins of peanut are heterogeneous and constitute only a small portion (about 1%) of the peanut protein. Of particular interest is the presence of two sets of polypeptides (b and c and d and e) differing slightly in their M_r's and pI's. In addition, the basic proteins appear to be unique in that they are rich in lysine and glycine, are low in acidic amino acids, and are glycosylated. Currently experiments are in progress to purify the major basic proteins of peanut seed and to identify the carbohydrate-containing polypeptide(s).

LITERATURE CITED

Andrews, P. *Biochem. J.* **1964**, *91*, 222.
Basha, S. M. M. *Plant Physiol.* **1979**, *63*, 301.
Basha, S. M. M.; Bazer, F. W.; Geisert, R. D.; Roberts, R. M. *J. Anim. Sci.* **1980**, *50*, 113.
Basha, S. M. M.; Beevers, L. *Plant Physiol.* **1976**, *57*, 93.
Basha, S. M. M.; Cherry, J. P. *J. Agric. Food Chem.* **1976**, *24*, 359.
Basha, S. M. M.; Pancholy, S. K. *Peanut Sci.* **1981**, *8*, 82.
Basha, S. M. M.; Pancholy, S. K. *J. Agric. Food Chem.* **1982**, *30*, 36.
Cherry, J. P.; Dechary, J. M.; Ory, R. L. *J. Agric. Food Chem.* **1973**, *21*, 652.
Davis, B. J. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404.
Dawson, R. *J. Nutr.* **1968**, *22*, 601.
Dechary, J. M.; Talluto, K. F.; Evans, W. J.; Carney, W. B.; Altschul, A. M. *Nature (London)* **1961**, *190*, 1125.
Horst, M. N.; Basha, S. M. M.; Baumbach, G. A.; Mansfield, E. D.; Roberts, R. M. *Anal. Biochem.* **1980**, *102*, 399.
Irving, G. W.; Fontaine, R. D.; Warner, R. C. *Arch. Biochem.* **1946**, *7*, 475.
Johns, C. O.; Jones, D. B. *J. Biol. Chem.* **1916**, *28*, 77.
Jones, D. B.; Horn, M. J. *J. Agric. Res. (Washington, D.C.)* **1930**, *40*, 672.
Koshiyama, I. *Agric. Biol. Chem.* **1966**, *30*, 646.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Neucere, N. J. *Anal. Biochem.* **1969**, *27*, 15.
Neucere, N. J.; Conkerton, E. J. *J. Agric. Food Chem.* **1978**, *26*, 683.
O'Farrell, P. Z.; Goodman, H. M.; O'Farrell, P. H. *Cell (Cambridge, Mass.)* **1977**, *12*, 1133.
Pancholy, S. K.; Deshpande, A. S.; Krall, S. *Proc. Am. Peanut Res. Educ. Assoc.* **1978**, *10*, 30.
Reisfield, R. N.; Lewis, U. J.; Williams, D. E. *Nature (London)* **1962**, *195*, 281.
Rondle, C. J. M.; Morgan, W. J. J. *Biochem. J.* **1955**, *61*, 586.
Shetty, K. J.; Rao, M. N. S. *Anal. Biochem.* **1974**, *62*, 108.
Tombs, M. P. *Biochem. J.* **1965**, *96*, 119.
Yemm, E. W.; Willis, A. J. *Biochem. J.* **1954**, *57*, 508.

Received for review May 3, 1982. Accepted August 10, 1982. This research was supported by a grant from the Cooperative State Research Service, U.S. Department of Agriculture, Washington, DC.

Effect of Succinylation on the Protein Quality and Urinary Excretion of Bound and Free Amino Acids

Maria Siu and Lilian U. Thompson*

The net protein ratio (NPR) of whey protein concentrate succinylated at four different levels was determined in order to establish an appropriate level of modification that will not adversely affect the protein quality. The utilization of the absorbed succinyl amino acids was also determined in rats by examining the urinary excretion of bound and free amino acids. Succinylated whey concentrate (SWC) with 37% succinylation is still a good quality protein with NPR higher than that of casein. At higher levels of succinylation, the NPR was adversely lowered. Rats fed SWC had high urinary nitrogen excretion, but little of the succinyllysine and none of the succinylcysteine and succinylthreonine were recovered as bound amino acids in the urine. Apparently these amino acids were partially catabolized to forms that were unavailable to the body. Further metabolic and toxicity studies of the succinyl amino acids are recommended.

Succinylation of heat-denatured cheese whey protein concentrates (WC) has been described as a means of improving the protein's functional properties and utilization in food systems (Thompson and Reyes, 1980). The potential use of such modified protein in food makes information on the nutritional and toxicological properties important. As reported previously (Siu and Thompson, 1982), succinylation affects the digestibility of amino acids. However, the relatively high in vivo amino acid digestibilities did not correspond with the poor in vitro results. For example, with exhaustively succinylated whey protein, there was a 98% decrease in in vitro lysine digestibility but only a 14% decrease in the in vivo value. The differences between in vitro and in vivo results were attributed to the absorption of succinylated amino acids or succinylated dipeptides in vivo.

The utilization of the succinylated amino acids or succinylated dipeptides depends on the ability of the cytoplasmic enzymes to release the dipeptides to individual succinylated or free amino acids and the ability of the organ's enzymes to deacylate the succinyl groups. Mammalian tissues contain acylases that act on a number of *N*-acyl amino acids (Endo, 1978; Paik and Benoiton, 1963), but their specific activities toward succinylated amino acids have not been reported. In a study of acylated methionine derivatives (Boggs, 1978), the specificity of the acylase appeared to be a function of the chemical group attached to methionine. The enzyme does not hydrolyze N^{α}-

Department of Nutritional Sciences, University of Toronto, Toronto, Canada M5S 1A8.

0021-8561/82/1430-1179$01.25/0

Table I. Weight Gain, Food Consumption, and Net Protein Ratio (NPR) of Rats Fed the Succinylated and Control Protein Diets[a]

diet	initial weight, g	weight gain, g	food consumption, g	NPR
CA	93.9 ± 3.6	45.0 ± 1.8^{u}	168.3 ± 6.8^{w}	3.57 ± 0.12^{v}
LA	93.0 ± 3.3	56.8 ± 2.4^{vw}	178.6 ± 3.3^{wx}	4.82 ± 0.13^{w}
WC	90.1 ± 1.9	65.4 ± 2.1^{x}	189.7 ± 6.4^{x}	4.90 ± 0.14^{w}
SWC.05	92.9 ± 1.9	63.4 ± 3.6^{vx}	181.4 ± 4.2^{wx}	4.94 ± 0.10^{w}
SWC.09	94.9 ± 3.3	50.8 ± 3.6^{uw}	174.6 ± 6.0^{wx}	4.23 ± 0.12^{x}
SWC.46	92.4 ± 1.0	0.9 ± 1.5^{y}	128.6 ± 5.2^{y}	2.04 ± 0.04^{y}
SWC2.04	93.2 ± 1.9	−16.6 ± 1.7^{z}	77.3 ± 5.4^{z}	1.10 ± 0.19^{z}
NF	93.0 ± 2.0	−25.2 ± 0.9^{z}	72.0 ± 3.4^{z}	

[a] CA = casein; LA = lactalbumin; WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively; NF = nitrogen free. Mean ± SEM: means with different superscripts in the same column are significantly different ($P < 0.05$).

succinylmethionine but is active toward other acylated derivatives. Like N^{α}-succinylmethionine, it is possible that other amino acids with succinylated ϵ-NH_2, SH, and OH groups are resistant to acylase; consequently, they may not be available as a source of amino acid.

Others have observed the excretion of bound lysine in the urine by rats fed propionyl-, acetyl-, and succinyllysine (Bjarnason and Carpenter, 1969; Groninger and Miller, 1979). It appears that the unhydrolyzable acylated lysine derivatives were not catabolized or retained by the body but were excreted as such in the urine. Urinary excretion of bound cysteine and threonine have not been examined previously. The objectives of this study were to determine the protein quality of the succinylated cheese whey protein concentrates and to determine the extent to which succinylation has affected the utilization of the amino acids by measurement of the urinary excretion of free and bound amino acids.

EXPERIMENTAL SECTION

Sample Preparation and Composition. Preparation of WC and the WC succinylated at levels of 0.05, 0.09, 0.46, or 2.04 g of succinic anhydride/g of WC (SWC.05, SWC.09, SWC.46, and SWC2.04), as well as proximate and amino acid composition, and the percent of bound ϵ-NH_2, SH, and OH groups of WC and the four SWCs have previously been reported (Siu and Thompson, 1982).

Rat Feeding Trial. The net protein ratio (NPR) was determined according to Lachance et al. (1977). Forty-eight male weanling rats of the Wistar strain (Woodlyn Farms, Ltd., Guelph, Ontario, Canada), 80–100 g of initial body weight, were divided into eight treatment groups. Each group was fed a 10% protein diet with the protein supplied by either WC, SWC.05, SWC.09, SWC.46, SWC2.04, casein (CA), or lactalbumin (LA) for 14 days. The diets were formulated according to AOAC (1980) specification and were given to the rats ad libitum. An additional treatment group was fed a nitrogen-free (NF) diet. Rats were housed in individual hanging, wire-meshed stainless steel cages in a room kept at 20–23 °C and on a 12-h (0800–2000 h) light–dark cycle. Weight gain and food intake were recorded 3 times a week. Net protein ratio (NPR) was calculated as the ratio of weight gain of test animal plus weight loss of animal fed the nonprotein diet to protein consumed by the test animal.

On day 7, rats were transferred to stainless steel metabolic cages that allowed separate collection of urine and feces. Urine was collected on days 13 and 14. One milliliter of 1 N HCl was added to the urine as a preservative, and the urine was kept frozen (−20 °C) until needed for analysis. The urine was pooled to provide two samples per treatment group, and the pooled urine volume was measured. Total nitrogen was determined by micro-Kjeldahl procedure (AOAC, 1980). Urine was prepared for amino acid analysis by mixing equal volumes of the urine and 15% sulfosalicylic acid, followed by centrifugation at 2500 rpm. Protein-free supernatant (400 μL) was injected into a Beckman amino acid analyzer, Model 120, for free amino acid determination. The protein-free supernatant was also subjected to acid hydrolysis (Blackburn, 1968), which released the conjugated amino acids into the free forms, and was reanalyzed for total amino acid content. The bound amino acids were calculated by subtracting the free from the total amino acid values. The amounts of free and bound amino acids excreted in 48 h were calculated, and the data were expressed as percent of ingested amino acid excreted either as free or as bound acids. Amino acid ingested was calculated from the protein consumption and the amino acid composition of the proteins shown in the previous paper (Siu and Thompson, 1982).

The growth data and the urine amino acid data of WC and the SWCs were compared to those of LA and CA, the reference proteins commonly used in nutritional studies. The data were subjected to analysis of variance and Duncan's multiple range test (Duncan, 1955).

RESULTS

NPR. Weight gain, food consumption, and the NPR values are presented in Table I. When the four SWC treatment groups were compared with the WC control group, there was a progressive lowering in weight gain and food consumption in rats fed proteins of increasing level of succinylation. Rats fed SWC.09, -.46, and -2.04 had significantly lower weight gain and rats fed SWC.46 and -2.04 had lower food consumption than WC. SWC.09, -.46, and -2.04 had lower NPR values than WC and SWC.05, which was due to the poorer weight gain and food consumption by rats fed these proteins. The low NPR of SWC.46 and SWC2.04 indicated their poor nutritional quality, disagreeing with an earlier report (Thompson and Reyes, 1980). The NPR values of SWC.09 was significantly lower than that of WC. Nevertheless, SWC.09 can still be considered as a good source of protein since it has higher NPR than CA. At a very low level of succinylation (SWC.05), no effect on the protein quality was observed. LA and WC had similar NPR as expected, since LA is the commercial whey protein preparation. WC and LA had higher NPR than CA, in agreement with the protein efficiency ratio and net protein utilization data of others (Forsum, 1974).

The growth data for the succinylated proteins were in agreement with that observed by Groninger and Miller (1979), Creamer et al. (1971), and Groninger (1973). Most authors attributed the low protein quality of succinylated proteins to the animal's inability to utilize succinyllysine, but the study by Creamer et al. (1971) on acetylcasein indicated that the availabilities of both lysine and the sulfur amino acids are affected. A progressive decrease in the NPR of succinylated proteins was observed as the level of modification increased (Figure 1) and can be re-

Table II. Nitrogen Excretion in Urine of Rats Fed the Succinylated and Control Protein Diets[a]

diet	mg of N excreted/2 days	% of ingested N excreted in 2 days
CA	432.40 ± 27.60^{w}	27.03 ± 2.03^{w}
LA	225.15 ± 12.94^{xy}	17.16 ± 0.28^{xy}
WC	237.50 ± 3.05^{xy}	15.95 ± 0.02^{x}
SWC.05	200.95 ± 3.03^{y}	20.88 ± 0.11^{xy}
SWC.09	294.53 ± 9.31^{x}	21.27 ± 0.70^{xy}
SWC.46	382.80 ± 14.15^{z}	50.39 ± 0.12^{z}
SWC2.04	266.73 ± 21.42^{xy}	57.52 ± 5.33^{z}

[a] CA = casein; LA = lactalbumin; WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively; NF = nitrogen free. Mean ± SEM: means with different superscripts in the same column are significantly different ($P < 0.05$).

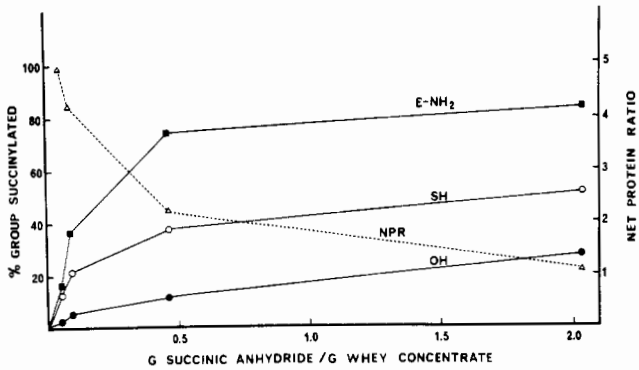

Figure 1. Net protein ratio (NPR) and level of modification of the succinylated proteins.

lated to the progressive increase in the amount of succinylated lysine, cysteine, and threonine in the modified proteins. Low level of succinylation (SWC.05) did not affect the protein quality because the modified protein still contained enough unsuccinylated lysine, cysteine, and threonine to meet the animal's requirement. This was verified by calculating the amount of unsuccinylated amino acids in the SWC.05 diet and comparing the values with the National Academy of Sciences (1978) published amino acid requirement of the rat.

Urinary Nitrogen and Amino Acids. Rats fed proteins with increasing level of succinylation excreted more nitrogen in the urine, with values from SWC.46 and -2.04 groups being significantly different from WC (Table II). SWC.46- and -2.04-fed groups excreted approximately 50% of the ingested nitrogen in the urine. Therefore, nitrogen that was apprently absorbed was not retained by the body but was excreted in the urine.

The percent of ingested amino acid excreted as free or as bound acid and the percent of total amino acid excreted in bound form in the urine are summarized in Tables III, IV, and V. The amino acid data obtained from rats fed the treated proteins (SWCs) were compared with the data from rats fed the high-quality proteins (WC, LA, and CA) by assuming that the amount of amino acids excreted from rats fed the latter were physiologically normal.

When the free amino acid data from rats fed the four SWCs were compared with those from those fed the three control diets, only the SWC.46-fed group excreted more of the amino acids ingested as free acids in the urine (with significantly higher threonine, valine, isoleucine, leucine, and phenylalanine excretion) (Table III). The percent of lysine ingested and excreted free in the urine of the SWC-fed groups was unaffected. This agrees with the urinary free lysine data of Bjarnason and Carpenter (1969) when rats were fed acetyl- and propionyllysine.

Rats fed the four SWCs excreted more of the ingested lysine in the bound form, with values from SWC.46- and SWC2.04-fed groups being significantly higher than those from rats fed the three control diets (Table IV). Furthermore, rats fed SWC.46 and -2.04 had significantly higher excretion of the ingested threonine, methionine, and leucine as bound acids and, for SWC2.04, higher bound

Table III. Percent of Ingested Amino Acid Excreted as Free Acid in Urine of Rats Fed Various Protein Sources[a]

amino acid	CA	LA	WC	SWC.05	SWC.09	SWC.46	SWC2.04
Lys	$0.08^{w} \pm 0.0$	$0.25^{xy} \pm 0.01$	$0.19^{wx} \pm 0.01$	$0.33^{y} \pm 0.01$	$0.30^{xy} \pm 0.01$	$0.28^{xy} \pm 0.07$	$0.17^{wx} \pm 0.0$
Thr	$0.46^{w} \pm 0.04$	$0.37^{w} \pm 0.02$	$0.39^{w} \pm 0.02$	$0.67^{w} \pm 0.0$	$0.48^{w} \pm 0.01$	$1.20^{x} \pm 0.26$	$0.73^{wx} \pm 0.07$
Val	$0.08^{w} \pm 0.02$	$0.16^{w} \pm 0.04$	$0.11^{w} \pm 0.0$	$0.11^{w} \pm 0.0$	$0.13^{w} \pm 0.02$	$0.47^{x} \pm 0.04$	$0.15^{w} \pm 0.03$
Cys	0	0	0	0	0	0	0
Met	0	0	0	0	0	0	0
Ile	$0.14^{w} \pm 0.01$	$0.29^{w} \pm 0.02$	$0.28^{w} \pm 0.01$	$0.36^{w} \pm 0.06$	$0.30^{w} \pm 0.06$	$0.65^{x} \pm 0.09$	$0.32^{w} \pm 0.07$
Leu	$0.09^{w} \pm 0.01$	$0.11^{w} \pm 0.01$	$0.11^{w} \pm 0.0$	$0.14^{w} \pm 0.02$	$0.15^{w} \pm 0.04$	$0.32^{x} \pm 0.03$	$0.18^{w} \pm 0.05$
Tyr	$0.10^{w} \pm 0.0$	$0.27^{x} \pm 0.03$	$0.20^{wx} \pm 0.0$	$0.24^{wx} \pm 0.02$	$0.16^{wx} \pm 0.0$	$0.30^{x} \pm 0.02$	$0.31^{x} \pm 0.08$
Phe	$0.08^{w} \pm 0.02$	$0.24^{wx} \pm 0.03$	$0.11^{w} \pm 0.01$	$0.25^{wx} \pm 0.03$	$0.15^{w} \pm 0.0$	$0.44^{x} \pm 0.02$	$0.27^{wx} \pm 0.10$

[a] CA = casein; LA = lactalbumin; WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively; NF = nitrogen free. Mean ± SEM: means with different superscripts in the same column are significantly different ($P < 0.05$).

Table IV. Percent of Ingested Amino Acid Excreted as Bound Acid in Urine of Rats Fed Various Protein Sources[a]

amino acid	CA	LA	WC	SWC.05	SWC.09	SWC.46	SWC2.04
Lys	$0.30^{w} \pm 0.06$	$1.05^{w} \pm 0.05$	$1.03^{w} \pm 0.15$	$3.28^{w} \pm 0.20$	$6.01^{wx} \pm 0.20$	$11.29^{x} \pm 0.48$	$12.22^{x} \pm 3.99$
Thr	$0.71^{wx} \pm 0.03$	$0.62^{wx} \pm 0.0$	$1.06^{w} \pm 0.24$	$0.50^{x} \pm 0.04$	$0.52^{x} \pm 0.04$	$1.63^{y} \pm 0.03$	$3.07^{z} \pm 0.05$
Val	$0.34^{wxy} \pm 0.01$	$0.54^{w} \pm 0.06$	$0.33^{wxy} \pm 0.04$	$0.19^{y} \pm 0.01$	$0.29^{xy} \pm 0.04$	$0.47^{wx} \pm 0.09$	$0.14^{y} \pm 0.0$
Cys	$4.31^{w} \pm 0.41$	$0.59^{x} \pm 0.04$	$1.04^{x} \pm 0.03$	$0.87^{x} \pm 0.01$	$1.18^{x} \pm 0.09$	$1.19^{x} \pm 0.05$	$1.23^{x} \pm 0.0$
Met	$0.59^{w} \pm 0.03$	$1.10^{wxy} \pm 0.04$	$0.94^{wx} \pm 0.08$	$1.60^{y} \pm 0.0$	$1.48^{xy} \pm 0.07$	$2.64^{z} \pm 0.15$	$3.41^{z} \pm 0.24$
Ile	$0.17^{w} \pm 0.0$	$0.15^{w} \pm 0.03$	$0.18^{w} \pm 0.03$	$0.04^{w} \pm 0.03$	$0.05^{w} \pm 0.03$	$0.34^{w} \pm 0.15$	$0.53^{x} \pm 0.04$
Leu	$0.17^{w} \pm 0.0$	$0.19^{w} \pm 0.01$	$0.18^{w} \pm 0.04$	$0.15^{w} \pm 0.01$	$0.18^{w} \pm 0.02$	$0.41^{x} \pm 0.08$	$0.48^{x} \pm 0.04$
Tyr	$0.07^{w} \pm 0.01$	$0.06^{w} \pm 0.02$	$0.41^{wx} \pm 0.20$	$0.25^{w} \pm 0.10$	$0.18^{w} \pm 0.02$	$0.42^{wx} \pm 0.18$	$0.90^{x} \pm 0.09$
Phe	$0.33^{w} \pm 0.10$	$0.76^{wx} \pm 0.25$	$0.47^{w} \pm 0.04$	$0.26^{w} \pm 0.01$	$0.79^{wx} \pm 0.09$	$0.35^{w} \pm 0.15$	$1.22^{x} \pm 0.0$

[a] CA = casein; LA = lactalbumin; WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively; NF = nitrogen free. Mean ± SEM: means with different superscripts in the same column are significantly different ($P < 0.5$).

Table V. Percent of Total Amino Acid Excreted in Bound Form in Urine of Rats Fed Various Protein Sources[a]

amino acid	CA	LA	WC	SWC.05	SWC.09	SWC.46	SWC2.04
Lys	$84.90^{w} \pm 1.58$	$80.70^{w} \pm 0.41$	$84.40^{w} \pm 2.63$	$90.80^{x} \pm 0.62$	$95.30^{xy} \pm 0.05$	$97.70^{y} \pm 0.49$	$98.70^{y} \pm 0.64$
Thr	$60.60^{wxy} \pm 3.27$	$66.60^{wxy} \pm 0.62$	$73.20^{wz} \pm 5.79$	$42.90^{x} \pm 2.23$	$52.20^{xy} \pm 2.49$	$57.20^{wxy} \pm 5.71$	$80.70^{z} \pm 1.91$
Val	$80.30^{w} \pm 7.28$	$76.50^{w} \pm 6.10$	$75.40^{w} \pm 2.64$	$63.70^{wx} \pm 0.45$	$68.40^{wx} \pm 5.76$	$49.20^{x} \pm 6.91$	$59.7^{wx} \pm 0.0$
Cys	100	100	100	100	100	100	100
Met	100	100	100	100	100	100	100
Ile	$61.10^{w} \pm 0.0$	$33.90^{wx} \pm 6.64$	$39.40^{wx} \pm 3.96$	$19.10^{x} \pm 0.0$	$12.80^{x} \pm 10.03$	$32.60^{wx} \pm 13.42$	$61.70^{w} \pm 6.83$
Leu	$63.80^{w} \pm 0.0$	$63.10^{w} \pm 3.94$	$61.20^{w} \pm 5.92$	$52.80^{w} \pm 7.88$	$56.70^{w} \pm 8.54$	$55.60^{w} \pm 7.23$	$73.10^{w} \pm 3.95$
Tyr	$40.58^{wx} \pm 3.08$	$17.50^{x} \pm 4.64$	$66.40^{wx} \pm 13.77$	$50.00^{wx} \pm 9.20$	$52.00^{wx} \pm 3.06$	$57.30^{wx} \pm 13.27$	$74.60^{w} \pm 6.55$
Phe	$82.40^{w} \pm 1.73$	$76.00^{wx} \pm 4.37$	$80.80^{w} \pm 0.06$	$50.00^{xy} \pm 4.10$	$83.80^{w} \pm 1.77$	$42.60^{y} \pm 12.49$	$81.60^{w} \pm 5.64$

[a] CA = casein; LA = lactalbumin; WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively; NF = nitrogen free. Mean ± SEM: means with different superscripts in the same column are significantly different ($P < 0.05$).

isoleucine excretion than rats fed WC, LA, and CA. Bound cysteine excretion by rats fed the four SWCs was similar to that of the controls.

Urine collected from the three control groups had similar ratios of bound to total amino acid (Table V). The ratios in the urine from WC- and CA-fed groups ranged from 40 to 85% and were similar to the 50–75% ratios found in the urine of casein-fed rats (Schweigert, 1947). Schweigert (1947) reported 70% bound threonine in the rat urine, similar to the ratio observed in this experiment. In contrast, a lower percent (40%) of bound lysine was observed by Sauberlich et al. (1948) in the urine of rats fed the casein diet.

The percent of total amino acid excreted in the bound form in the urine of the SWC-fed rats varied and were in general lower than in the three control groups. Lysine was the only amino acid with a progressively higher ratio as rats were fed proteins of increasing level of succinylation. The fluctuation in the urinary amino acid values may be related to individual variation in amino acid excretion and the different quantity of urine voided by the rats within the 48 h.

DISCUSSION

More amino acids were excreted in the urine when the rats were fed the highly succinylated proteins. This was reflected in the greater percent of ingested amino acids excreted in free and bound forms for the SWC.46 treatment group and the greater percent of ingested amino acids excreted in bound forms for the SWC2.04 group. The larger quantity of urinary amino acids in SWC.46 and SWC2.04 groups was related to feeding rats with diets that contain unavailable essential amino acid such as lysine. Increased urinary amino acid excretion was reported by Sauberlich et al. (1948) and Schweigert (1947) when rats were fed amino acid deficient diets.

Progressively more bound than free lysine was excreted in the urine from rats fed proteins of increasing levels of succinylation. This is probably due to the excretion of some succinyllysine in the urine as observed by Groninger and Miller (1979).

In the experiment by Bjarnason and Carpenter (1969) in which rats were fed either propionyllysine or acetyllysine, 0 and 50% of the ingested propionyllysine and acetyllysine, respectively, were found to be utilized for growth, while 41 and 18% of the ingested propionyl- and acetyllysine, respectively, were excreted as such in the urine. Groninger and Miller (1979) also showed that the absorbed and unmetabolized [^{14}C]succinyllysine was primarily excreted in the urine as succinyllysine. In this experiment, rats fed SWC 2.04 excreted in the urine 12% of the ingested lysine as bound lysine. On the basis of this data, it appears that the majority of the absorbed lysine was utilized by the body. However, weight loss was observed for this group of animals. The urinary bound lysine content was low when compared with other experimental values and was too low to account for the weight loss by these rats. In this experiment, the rats were kept on the succinylated proteins for 12 days prior to urine collection, while Groninger and Miller (1979) used a 24-h assay. The rats may have adapted to the diets after being fed for a longer time period and may have metabolized succinyllysine partially to a form that was nonutilizable and was unidentifiable by the methodology of this experiment. Stephens et al. (1977) also recovered little of the absorbed N^{α}-palmitoyllysine in the urine of rats fed for 10 days. These researchers postulated that the lysine derivative may be either retained or catabolized in a manner that destroyed rather than released the lysine moiety.

Lysine can be catabolized by two pathways (Lehninger, 1975). In one pathway lysine condenses with α-oxoglutarate to yield saccharopine. This step involves reaction with the free ϵ-NH_2 group of lysine. Saccharopine is converted to glutamate and α-aminoadipic acid semialdehyde, which is then ultimately converted to acetoacetyl-CoA. In another pathway the α-amino group of lysine is oxidized, presumably by L-amino acid oxidase, to yield α-oxo-ϵ-aminocaproic acid. The metabolite spontaneously cyclizes to Δ-piperidine-2-carboxylic acid and then is metabolized to α-aminoadipic acid semialdehyde and ultimately to acetoacetyl-CoA.

The second pathway is not the major catabolic pathway for L-lysine as shown by radioactive studies (Grove et al., 1969; Krebs, 1964). In an in vitro test, the amino acid oxidase was found to attack lysine at a very slow rate, but it attacked ϵ-acetyllysine and other lysine derivatives readily (Neuberger and Sanger, 1944). The authors postulated that the free terminal amino group inhibited the oxidase due to its basic character, and by masking the ϵ-amino group, oxidation at moderate rate could take place. It has been postulated by a number of researchers that the first metabolite in this pathway is N^{ϵ}-acetyl-L-lysine (Paik and Benoiton, 1963; Kim et al., 1964; Meister, 1954). The compound is then deaminated by the oxidase to give α-oxo-ϵ-acetamidocaproic acid, which would then be deacetylated by ϵ-lysine acylase to give Δ-piperidine-2-carboxylic acid (Paik and Benoiton, 1963).

In this experiment, succinyllysine was probably not metabolized through saccharopine pathway since its ϵ-amino group was blocked from reacting with α-oxoglutarate. However, when the ϵ-amino group was blocked, succinyllysine was attacked by amino acid oxidase to give

α-oxo-ε-(succinylamido)caproic acid. This may not be metabolized further if the acylase cannot deacylate the succinyl group. Due to the slow rate of oxidative deamination reaction, some of the succinyllysine overflowed and was excreted in the urine.

Succinylcysteine and succinylthreonine did not appear to be excreted in the urine since the ratio of bound to total threonine and the percent of ingested excreted as bound cysteine were unaffected. Possibly, the acylase hydrolyzed and released the bound cysteine and threonine to free forms, or they were partially catabolized or were retained in the body. Further studies are necessary before the bioavailabilities of succinylcysteine and succinylthreonine can be concluded.

One of the catabolic pathways for cysteine involves transamination of the α-amino group to form mercaptopyruvic acid followed by the elimination of the SH group to give pyruvic acid (Lehninger, 1975). Succinylcysteine may undergo transamination of the α-amino group but may not be able to convert to pyruvic acid because its SH group is blocked by succinate. Threonine can be catabolized via aldol cleavage to yield glycine and acetaldehyde (Lehninger, 1975). Succinylthreonine may be able to catabolize to glycine, acetaldehyde, and succinic acid.

If the succinyl amino acids can only be partially metabolized or if they were retained in the body, then the possible toxic effect of these substances to the body need to be examined. The metabolic fate of these succinyl amino acids can be further studied by using labeled succinyl amino acids.

Only one toxicity test on an acylated protein (acetylcasein) has been reported in literature (Creamer et al., 1971). Mice fed acetylcaseine were lighter in weight and had smaller litters than mice fed casein. However, mice fed acetylcasein for three generations did not develop histological changes in their organs. Succinyllysine may behave differently from acetyllysine since the latter can apparently be catabolized via the amino acid oxidase pathway. Long-term dose–response feeding studies with accompanying histopathological analyses of body tissues are needed to assess the suitability of using succinylated proteins in food systems.

The NPR data suggested that whey protein concentrates can be succinylated to a low level (with 15% ε-NH_2 groups succinylated) without affecting the protein quality. A medium level of succinylation (with 37% ε-NH_2 groups succinylated) can still produce a good-quality protein with higher NPR than casein. High levels of succinylation (with 74–83% ε-NH_2 succinylated groups) have been found to have deleterious effects on protein quality.

The functional properties of highly succinylated whey protein (with 70% bound ε-NH_2 groups) are good (Thompson and Reyes, 1980). Improved functional properties have also been reported when proteins were succinylated to 30–55% (percent bound ε-NH_2) (Eisele and Brekke, 1981; Choi et al., 1981; Franzen and Kinsella, 1976; Ball et al., 1979). This suggests that whey protein concentrates can be succinylated to a medium level with little loss in nutrititive value and with possible improvements of functional properties. However, functional properties of whey proteins succinylated to low and medium levels remain to be determined. These modified proteins can be used in food systems such as meat analogues since these foods may be consumed in large quantities and the nutritional properties may be important. The highly succinylated protein can be used as a functional ingredient in systems where the nutritional properties of the succinylated protein is unimportant, such as in salad dressings and coffee whiteners. Performance of SWCs in these products proved to be good (Thompson and Reniers, 1982).

ACKNOWLEDGMENT

We thank L. Baker and C. Leprohon for technical assistance and M. Baigent and A. V. Rao for helpful advice.

LITERATURE CITED

AOAC "Official Methods of the Association of Official Analytical Chemists"; AOAC: Washington, DC, 1980.
Ball, H. R., Jr.; Winn, S. E.; King, A. J.; Palladino, D. K. *Poult. Sci.* **1979**, *58*, 1034.
Bjarnason, J.; Carpenter, K. J. *Br. J. Nutr.* **1969**, *23*, 859.
Blackburn, S. "Amino Acid Determination Methods and Techniques"; Marcel Dekker: New York, 1968.
Boggs, R. W. In "Nutritional Improvement of Food and Feed Proteins"; Friedman, M., Ed.; Plenum Press: New York, 1978; pp 571–586.
Choi, Y. R.; Lusas, E. W.; Rhee, K. C. *J. Food Sci.* **1981**, *46*, 954.
Creamer, L. K.; Roeper, J.; Lohrey, E. H. *N.Z. J. Dairy Sci. Technol.* **1971**, *6*, 107.
Duncan, B. D. *Biometrics* **1955**, *11*, 1.
Eisele, T. A.; Brekke, C. J. *J. Food Sci.* **1981**, *46*, 1096.
Endo, V. *Biochim. Biophys. Acta* **1978**, *523*, 207.
Forsum, E. *J. Dairy Sci.* **1974**, *57*, 665.
Franzen, K. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1976**, *24*, 788.
Groninger, H. S. *J. Agric. Food Chem.* **1973**, *21*, 978.
Groninger, H. S.; Miller, R. *J. Agric. Food Chem.* **1979**, *27*, 949.
Grove, J. A.; Gilbertson, T. J.; Hammerstedt, R. H.; Henderson, L. M. *Biochim. Biophys. Acta* **1969**, *184*, 329.
Kim, S.; Benoitor, L.; Paik, W. K. *J. Biol. Chem.* **1964**, *239*, 3790.
Krebs, H. A. In "Mammalian Protein Metabolism"; Munro, H. N.; Allison, J. B., Eds.; Academic Press: New York, 1964; Vol. I, pp 125–170.
Lachance, P. A.; Bressani, R.; Elias, L. *Food Technol. (Chicago)* **1977**, *31* (6), 82.
Lehninger, A. L. "Biochemistry"; Worth Publishers: New York, 1975.
Meister, A. *J. Biol. Chem.* **1954**, *206*, 577.
National Academy of Sciences "Nutrient Requirements of Laboratory Animals"; National Academy of Sciences: Washington, DC, 1978.
Neuberger, A.; Sanger, F. *Biochem. J.* **1944**, *38*, 119.
Paik, W. K.; Benoiton, L. *Can. J. Biochem. Physiol.* **1963**, *41*, 1643.
Sauberlich, H. E.; Pearce, E. L.; Baumann, C. A. *J. Biol. Chem.* **1948**, *175*, 29.
Schweigert, B. S. *Proc. Soc. Exp. Biol. Med.* **1947**, *66*, 315.
Siu, M.; Thompson, L. U. *J. Agric. Food Chem.* **1982**, *30*, 743.
Stephens, C. A.; Veen-Baigent, M. J.; Anderson, G. H. *Can. J. Physiol. Pharmacol.* **1977**, *55*, 434.
Thompson, L. U.; Reniers, D. *J. Dairy Sci.* **1982**, in press.
Thompson, L. U.; Reyes, E. S. *J. Dairy Sci.* **1980**, *63*, 715.

Received for review February 22, 1982. Accepted July 6, 1982.