different species are presented in Table V. The results show that E:N, E:P, and E:T ratios are highest in the case of FPC made from picked ribbonfish, followed closely by catfish and milkfish. This may be the reason for higher PER and NPR values for FPC from picked ribbonfish and catfish.

Picked ribbonfish FPC has also given the highest value for EAAI, chemical score, and BV, which further confirms the higher PER and NPR values obtained in its case. The EAAI for the specie picked ribbonfish approaches very closely the EAAI for egg taken as 100.

Chemical Composition. Results on the distribution of chemical constituents in FPC from different species (Table VI) showed a minimum amount of moisture and a neglegible amount of crude fat present in the samples. The ash content is lower in FPC prepared from the picked ribbonfish specie.

Acceptability of the Product. Sensory evaluation showed that bread containing 5% FPC was as acceptable with respect to texture and flavor as bread with no FPC to a panel of judges. The results agree very well with those obtained by Sidwell et al. (1970), Revanker et al. (1965), Lahiry et al. (1962). The product was quite stable and there was no reversion of flavor when stored in glass jars with screw caps at 20–30 °C for more than a year. The product was free of *Escherichia coli*, *Salmonella*, and pathogenic anaerobes (total plate count below 2000/g).

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Some Chemical and Nutritional Properties of Acylated Fish Protein

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Data are presented on some of the chemical and nutritional properties of acylated fish protein. These include the extent of sulfhydryl group reaction, amino acid composition, protein efficiency ratio (PER) values, the rates of enzyme digestion, and the utilization of ^{14}C-labeled derivatives by rats. The PER of highly acetylated (about 75%) myofibrillar protein was 85% that of reference casein and the PER of medium succinylated (about 50%) myofibrillar protein was 73% that of reference casein. The feeding of ^{14}C-labeled acetyllysine and ^{14}C-labeled acetylated protein to rats showed that both free and protein-bound acetyllysine were partially utilized. ^{14}C-labeled succinyllysine and the succinyllysine in an enzyme hydrolysate of ^{14}C-labeled lysine protein, both administered intravenously were not utilized. Some utilization of orally administered free and protein-bound succinyllysine may have occurred; however, these utilization values may have been distorted by gut microbial activity.

The preparation and functional properties of acylated fish proteins have been described (Groninger, 1973; Groninger and Miller, 1975; Chen et al., 1975; Miller and Groninger, 1976). Kruckenberg (1956) was granted a patent on the production of acyl lysines for animal feed supplements. Also, White and Britton (1959) were granted a patent on the use of ϵ-acyl lysine derivatives in baked products. Continued interest in these free and protein-bound lysine derivatives as food materials makes information on the chemical and nutritional properties of these derivatives increasingly important.

The chemical properties of acylated proteins depend on the kinds of derivatives that are formed when a protein is reacted with an acylating agent. Acylating agents can react with the amino, thiol, hydroxyl, phenolic, and imidazole groups of protein. The main reaction is with the amino groups of protein (Grant-Green and Friedberg, 1970). Reaction with protein thiol groups has been reported by Habeeb et al. (1958), Hass (1964), Mühlrad et al. (1968), and Meighen and Schachman (1970). Reaction with protein hydroxy groups has been reported by Gounaris and Perlmann (1967), Brattin and Smith (1971),

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Nakamura and Fujita (1972), and Allen and Harris (1976). The formation of *O*-acetyltyrosine in protein has been described by Riordan and Vallee (1963) and Karibian et al. (1968). Riordan and Vallee (1964) and Gounaris and Perlmann (1967) have reported the formation of *O*-succinyltyrosyl residues in protein and their spontaneous deacylation. However, Nakamura and Fujita (1972) reported that none of the tyrosyl residues of succinylchymotrypsin were modified and Gounaris and Ottesen (1965) did not observe *O*-succinyltyrosyl residues in succinylated subtilopeptidase. Mühlrad et al. (1968) reported that succinylation of actin did not affect tyrosine or histidine residues. The work of Allen and Harris (1976) suggests that the deacylation of *N*-acylimidazole in a protein might be very rapid: the half-life of the hydrolysis of *N*-succinylimidazole at pH 8.5 was estimated to be about 1 min.

There have been few reports on the metabolism, nutrition, and toxicology of acylated protein derivatives. Animal deacylase capability has been studied by Paik and Benoiton (1963), Leclerc and Benoiton (1968), and Endo (1978). The nutritional availability of acyl derivatives of lysine has been estimated by Bjarnason and Carpenter (1969a), Boggs (1977), and Finot et al. (1977b). Creamer et al. (1971) reported PER values for acetyl- and succinylcasein and Belikow et al. (1975) obtained nutritive values for acetyl- and succinylcasein with the use of *Tetrahymena pyriformis*. Creamer et al. (1971) fed mice a diet in which most of the dietary protein was derived from acetylcasein; he reported that these animals survived and reproduced, but that the animals were lighter in weight, had smaller litters, and had less depot fat than control animals.

The objective of the work reported here is to examine a number of aspects of acylated fish protein relative to their suitability as foods. These include the estimate of the extent of the reaction of acylating agents with sulfhydryl groups; determination of protein efficiency ratios (PER) for protein acylated at various levels; estimates of the in vitro digestibility of acylated proteins; and determination of the utilization of acylated protein and lysine derivatives through the feeding of carbon-14 derivatives to rats.

MATERIALS

Fish. Rockfish (*Sebastes* sp.) fillets were obtained from commercial sources; fillets were used to prepare myofibrillar protein.

Rats. Male Sprague-Dawley rats, weighing about 200 g, were purchased from Tylor Labs., Bellevue, WA.

METHODS

Available Lysine. Amino groups were measured by a modification of the method of Fields (1972). Picryl chloride at a concentration of 100 mg/0.4 mL of water was added in 50-μL aliquots at 30-s intervals to the protein solution. The reaction was stopped after 10 min by the addition of 2.5 mL of sulfite–phosphate buffer. Absorbancy was measured at 420 nm.

Sulfhydryl. Sulfhydryl was estimated by the method of Janolino and Swaisgood (1975). The percent of sulfhydryl reacted was calculated from the amount of sulfhydryl found in the unacylated and acylated samples.

Nitrogen. Kjeldahl method was used as described in the AOAC (1975).

Protein. Protein was measured by the method of Lowry et al. (1951) and was estimated in chromatographic eluates by absorbancy measurements at 280 nm.

PER. The rat assay for PER was conducted according to methods outlined in the AOAC (1975).

Amino Acid Analysis. An ion-exchange chromatographic method was used; protein was hydrolyzed for 24 h in 6 N HCl at 110 °C; serine was increased by 10% and threonine by 5% to compensate for destruction by acid; half-cystine was determined after performic acid oxidation and hydrolysis and was calculated from the cysteic/alanine ratio. Tryptophan was determined after a 48-h alkaline hydrolysis at 135 °C (Hugli and Moore, 1972) and was calculated from the tryptophan/histidine ratio.

In Vitro Enzyme Hydrolysis. Peptic hydrolysis was carried out by the addition of 20 mg of pepsin (2760 units/mg) to a suspension of about 700 mg of protein that had been adjusted to pH 1.5–2.0 with HCl and maintained at a temperature of 37 °C. After 4 h in a water bath–shaker, the reaction was stopped by addition of 20% trichloroacetic acid (Cl_3CCOOH).

Tryptic hydrolysis was carried out by the addition of 4 mg of trypsin (9600 BAEE units/mg) to a suspension of about 700 mg of protein, in pH 8.0 borate buffer containing 0.1 M $CaCl_2$, maintained at 37 °C. After 4 h in a water bath–shaker, the reaction was stopped by addition of 20% Cl_3CCOOH.

Preparation of Unlabeled and ^{14}C-Labeled Acylated Proteins. Myofibrillar protein was prepared from rockfish flesh; subsequently, this protein was used to prepare unlabeled acylated protein essentially as described by Groninger (1973). The sodium salt of the acylated derivative was prepared in each instance. The derivative was freeze-dried.

The ^{14}C-labeled succinyl derivative was prepared by adding dropwise warm methyl Cellosolve containing 4.5 g of unlabeled succinic anhydride plus 50 μCi of [1,4-^{14}C]succinic anhydride into 600 mL of salt-solubilized protein which was stirred and held at 0 °C and the pH was maintained in the range of 8.0–8.5 with NaOH. Approximately 50% of the available lysine in this derivative was reacted with the anhydride.

The ^{14}C-labeled acetylated derivative was prepared by adding dropwise a mixture containing 1.5–2.0 mL of unlabeled acetic anhydride plus 50 μCi of [1-^{14}C]acetic anhydride into 300 mL of salt-solubilized myofibrillar protein which was stirred and held at 0 °C and the pH was maintained in the range of 8.0–8.5 with NaOH. Approximately 60% of the available lysine in this derivative was reacted with anhydride.

Both the succinyl and acetyl derivatives were purified as follows: 2–3 g of sodium acetate or sodium succinate was added to the acetyl and succinyl derivatives, respectively. They were precipitated with 0.1–1.0 N HCl at a pH of 4.0–4.2, and the precipitates were recovered by centrifugation. They were then resuspended, neutralized with NaOH, and precipitated a second time. The precipitates were extracted three times with hot isopropyl alcohol to remove fat. Residual alcohol was removed with three water washes. The derivatives was neutralized with NaOH and dialyzed for 72 h at 0 °C against 0.1 N NaCl solution that was changed a total of five times. The Cl_3CCOOH filtrates showed little or no Cl_3CCOOH-soluble radioactivity. The specific activities for the acetylated and succinylated proteins were 1385 and 1000 cpm/mg of protein, respectively.

Preparation of Labeled Lysine Derivative. L-N^{ϵ}-[*acetyl*-1-^{14}C]Lysine was prepared using the method of Benoiton and Leclerc (1965). The melting point of the product was 228–229 °C; the reference value was 231 °C. It had an R_f similar to that of a commercial sample when analyzed on thin layers of cellulose with the solvent butanol–acetic acid/water (4:1:1) and visualized with nin-

hydrin. Calculated for $C_8H_{16}N_2O_3$: C, 51.1; H, 8.5; N, 14.9. Found: C, 50.87; H, 8.83; N, 14.77 (Galbraith Labs., Knoxville, TN). Radiopurity was established by chromatography on cellulose where all radioactivity chromatographed with the product. The product had a specific activity of 850 cpm/mg.

L-N^{ϵ}-[*succinyl*-1,4-^{14}C]Lysine was prepared using a variation of the method of Neuberger and Sanger (1943). Details of this preparation will be published elsewhere. The melting point of the product crystallized from ethanol was 227–229 °C. Succinyllysine migrated on thin layers of cellulose with the solvent methanol/water/pyridine (20:5:1) as a single component when visualized with ninhydrin. Calculated: C, 48.8; H, 7.32; N, 11.4. Found: C, 48.52; H, 7.31; N, 11.33 (Galbraith Labs., Knoxville, TN). Radiopurity was established by chromatography on cellulose where all radioactivity chromatographed with the product. The product had a specific activity of 8900 cpm/mg. Gounaris and Ottesen (1965) succinylated DL-lysine for use as a chromatographic standard; however, neither the method of purification nor its properties were reported.

Enzyme-Hydrolyzed Succinylated Protein Preparation. A 10-mL suspension of succinylated protein was hydrolyzed using a combination of pepsin and Pronase (0.1 g) at a neutral pH and a temperature of 20 °C. The hydrolysate was separated from unreacted protein and enzyme by passing it through an Amicon ultrafilter membrane, type PM-10. The specific activity of the hydrolysate was 2337 cpm/mg of N.

Treatment of the Rats. Rats were fed Purina Laboratory Chow and were housed in wire-bottom cages. Animals were fasted 24 h prior to and during the experiment, but water was allowed ad libitum. Animals weighing 200–250 g were given [^{14}C]acyl derivatives as follows: acylated protein and acyllysine by stomach tube and acyllysine intravenously, in the tail while the animal was slightly anesthetized with diethyl ether. The rats were placed in individual metabolism cages where CO_2 and excreta were collected for 24 h. The rats were sacrificed and the organs removed and frozen.

Measurement of Radioactivity in CO_2, Excreta, and Organs. Expired air was bubbled through hyamine hydroxide, followed by a 1:1 phenethylamine–MeOH solution. These CO_2-absorbing solutions were changed three or four times during the 24-h period. Aliquots of these solutions were mixed with a toluene–PPO–POPOP scintillation reagent and counted. Urine was collected periodically during the 24-h day and counted directly by mixing with phase combining system (PCS) scintillation cocktail. Rat organs and feces were solubilized with Soluene, decolorized with H_2O_2, and counted after mixing with Dimilume-30 scintillation reagent. Alternatively, rat organs and feces were dried, and combusted, and the CO_2 was collected in hyamine hydroxide and counted after mixing with a toluene–PPO–POPOP scintillation reagent.

Fractionation of Urine Compounds. The radioactive components from the urines of rats administered ^{14}C acylated protein or lysine were separated from the nonradioactive components by use of gel filtration on Sephadex G-10 followed by cellulose thin-layer chromatography.

Each 24-h urine sample was concentrated to 2–4 mL. Gel filtration was carried out by applying a 2-mL aliquot of the concentrated urine to a column of G-10-20 (58 × 1.5 cm) and eluting at a flow rate of 0.3 mL/min with 0.1 M acetic acid saturated with chloroform (Hamilton and Lou, 1972; Lou and Hamilton, 1973; Lou, 1975). Eluted activity was measured using a PSC cocktail.

Table I. Reaction of Acylating Agents with Sulfhydryl Groups at Various Levels of Acylation

protein	extent of acylation, %	SH groups reacted, %
unacylated protein	0	0
acetylated protein	31	24
	57	22
	85	17
succinylated protein	23	32
	45	33
	75	35

The radioactive eluate from the G-10 gel filtration was concentrated and aliquots of approximately 40 μL applied to Whatman K2F cellulose plates. The plates were developed in butanol/acetic acid/water (63:27:10) and stained with a ninhydrin–cadmium spray. To obtain larger amounts of material for use in composite analysis, 500 μL of concentrated eluate was applied in the form of a band on a 20 × 20 cm plate and chromatographed. The components on the developed chromatogram were located by spraying only a narrow vertical segment of the plate with the ninhydrin–cadmium. The unstained bands were scraped from the plates and eluted with 3 mL of 10% propyl alcohol, and the eluate was counted using a PCS cocktail. The eluates of bands that contained radioactivity were concentrated, hydrolyzed, and their amino acid composition determined using ion-exchange chromatography. Alternatively, the eluates of bands that contained radioactivity were concentrated and hydrolyzed, and the amino acids were tentatively identified after chromatography on cellulose plates with the solvent system used for unhydrolyzed material.

RESULTS AND DISCUSSION

Results of Acylation Agents with Sulfhydryl Groups of Protein. When myofibrillar protein was succinylated, about one-third of the sulfhydryl groups were reacted; acetylation resulted in reaction of up to about one-fourth of the sulfhydryl (Table I). At higher levels of acetylation, apparently some deacylation of sulfhydryl occurred. The extent of the reaction of succinic anhydride with sulfhydryl did not appear to be related to the extent of acylation of amino groups of protein.

Amino Acid Composition. The amino acid compositions of unacylated, acetylated, and succinylated proteins are given in Table II. The low value for methionine in the unacylated protein and the low value for half-cystine in the succinylated protein might be explained if it is assumed that some protein fractions of these mixtures were somehow washed out or eliminated during the process steps subsequent to acylation. The lower values for lysine in the acetylated and succinylated proteins might be attributed to incomplete deacylation of lysine during acid hydrolysis. Examples of the resistance of this amide bond to hydrolysis are given in the work of Gounaris and Ottesen (1965) who reported the presence of lysine-containing material in hydrolysates of succinylated subtilopeptidase and Gounaris and Perlmann (1967) who reported the presence of lysine-containing material in hydrolysates of DNP-succinyl pepsinogen.

PER Studies. Protein efficiency ratios were determined for the acylated protein to obtain an overall measure of their nutritional value (Table III). In general, acylation of the amino groups of a protein resulted in a decrease in the PER and in the feed efficiency. There tended to be a direct relation between the extent of acylation and the decrease in nutritive value; lower levels of acylation,

Table II. Amino Acid Composition of Unacylated, Acetylated, and Succinylated Myofibrillar Protein from Rockfish

amino acid	unacylated, g/16 g of N	acetylated, g/16 g of N	succinylated, g/16 g of N
alanine	4.98	4.91	4.76
arginine	6.35	6.14	6.02
aspartic acid	10.12	9.80	9.62
half-cystine	0.818	0.832	0.684
glutamic acid	16.89	16.04	15.82
glycine	3.04	2.98	2.93
histidine	2.06	2.03	1.98
isoleucine	4.78	4.67	4.54
leucine	8.18	7.77	7.68
lysine	10.67	8.99	9.05
methionine	2.60	3.20	3.23
phenylalanine	3.63	3.61	3.47
proline	3.44	3.32	3.77
serine	3.89	3.83	3.77
threonine	4.74	4.73	4.50
tryptophan	1.37	1.25	1.16
tyrosine	3.85	3.79	3.69
valine	4.85	4.89	4.44
total	96.18	92.80	90.71

Table III. Protein Efficiency Ratio and Feed Efficiency Values for Unacylated and Acylated Fish Proteins

protein	feed efficiency, g of feed/g of gain	PER, %
casein	3.7	100
unacylated fish protein	3.7	101
medium acetylated protein (about 50%)	3.6	103
highly acetylated protein[a] (about 75%)	4.4	85
low succinylated protein (about 25%)	3.9	96
medium succinylated protein[a] (about 40%)	5.1	73

[a] Statistically significant at the 5% level.

acetylation at approximately the 50% level, and succinylation at approximately the 25% level resulted in little or no loss in nutritive value. Acetylated proteins gave better growth than the succinylated derivative at about the same level of acylation.

There is a limitation in the usefulness of the PER test when it is used to measure the unavailability of an amino acid such as lysine that is generously supplied in fish protein. In this use, the PER will not detect the unavailability of lysine in that proportion of total protein lysine that is in excess of the dietary needs of the test animal.

There is general agreement between these results and those reported in the literature where similar protein and amino acid derivatives were tested for their nutritive value using a variety of test procedures. Bjarnason and Carpenter (1969a) found that N^{ϵ}-acetyllysine had a growth response of 50% that of free lysine. Also, acetylated bovine plasma albumin, 75–82% acetylated, gave a lower growth response than unmodified protein. This work was done using rats that were fed a lysine-deficient diet.

Creamer et al. (1971) reported that the PER of acetylcasein was 64% that of casein, and the addition of 0.9% lysine to the diet with acetylcasein only increased this PER to 80% that of casein. Acetylated whey protein had a PER that was 80% that of casein and the PER of succinylcasein was 12% of the reference, casein. The extent of acylation was not reported for these derivatives. However, if the reported ratios of anhydride and protein reactants are taken into account, it can be assumed that acylation of the ϵ-amino groups were nearly complete.

Table IV. Percentage of Trichloroacetic Acid Soluble Nitrogen after Peptic and Tryptic Treatment of Unacylated and Acylated Fish Protein

protein	pepsin[a] treatment	trypsin[b] treatment
unacylated protein	94	69
medium acetylated protein	95	29
medium succinylated protein	95	47

[a] 2.9% pepsin; incubated at 37 °C for 4 h. [b] 0.5% trypsin; incubated at 37 °C for 4 h.

Belikow et al. (1975), using the protozoan *Tetrahymena pyriformis* as the test animal, reported that acylation of the hydroxyl groups of casein did not reduce the nutritive value, whereas acylation of amino groups of casein led to a reduction of nutritive value. They obtained nutritive values of 48 and 12% for acetyl- and succinylcasein, respectively, compared to casein. The casein derivatives were acetylated at the 35% level and succinylated at the 74% level. Leclerc and Benoiton (1968) explained differences in nutrition values for mammals on the basis that they lack a deacylase that will hydrolyze acyl groups larger than acetyl or formyl from the ϵ nitrogen of lysine. This has been cited by a number of other authors (Bjarnason and Carpenter, 1969a,b; Creamer et al., 1971) as a reason to expect less utilization of derivatives that have a chain length greater than two.

Enzyme Hydrolysis of Acylated Proteins. In vitro proteolysis tests were conducted to obtain estimates of the rates of enzymic hydrolysis of acylated proteins. The relative rates of peptic and tryptic hydrolysis of unacylated and acylated protein are compared in Table IV. Pepsin hydrolyzed both succinylated and acetylated protein at the same rate as unacylated protein. Tryptic hydrolysis, however, was less for the succinylated protein and less for the acetylated protein. This reduced hydrolysis is consistent with the findings of Li and Bertsch (1960) that trypsin does not hydrolyze peptide bonds that involve acylated lysine. Varnish and Carpenter (1975), using a technique based on the analysis of the ileal contents of the chick, showed that propionylated lactalbumin had a digestibility of 0.79 as compared to 0.9 for the unacylated lactalbumin. However, they concluded that this reduced digestibility was only a partial answer to the reduced availability of propionyllactalbumin.

Feeding of ^{14}C Acylated Derivatives. *A. Distribution of Recovered Radioactivity.* The extent of recovery of $^{14}CO_2$ and radioactivity in the urine of rats fed ^{14}C acetylated and ^{14}C succinylated derivatives was used as a measure of the utilization of these materials. An example of this reasoning is given by Erbersdobler (1976) who described ϵ-fructoselysine as an unavailable compound and showed that when carbon-14-labeled fructoselysine was fed to rats as much as 86–92% of the administered radioactivity was recovered in the urine within 12 h. The 24-h recovery of radioactivity was 87% and 93% for intravenously administered succinyllysine and hydrolyzed succinylated protein, respectively. The percent recovery of orally administered succinyllysine was 75% and acetylated protein was 72%. The recovery of succinylated protein was not measured.

The distribution of radioactivity between $^{14}CO_2$ and urine during the 24-h period is given in Figure 1. In general, the acetyl derivatives were utilized at a greater level than the succinyl derivatives as indicated by the

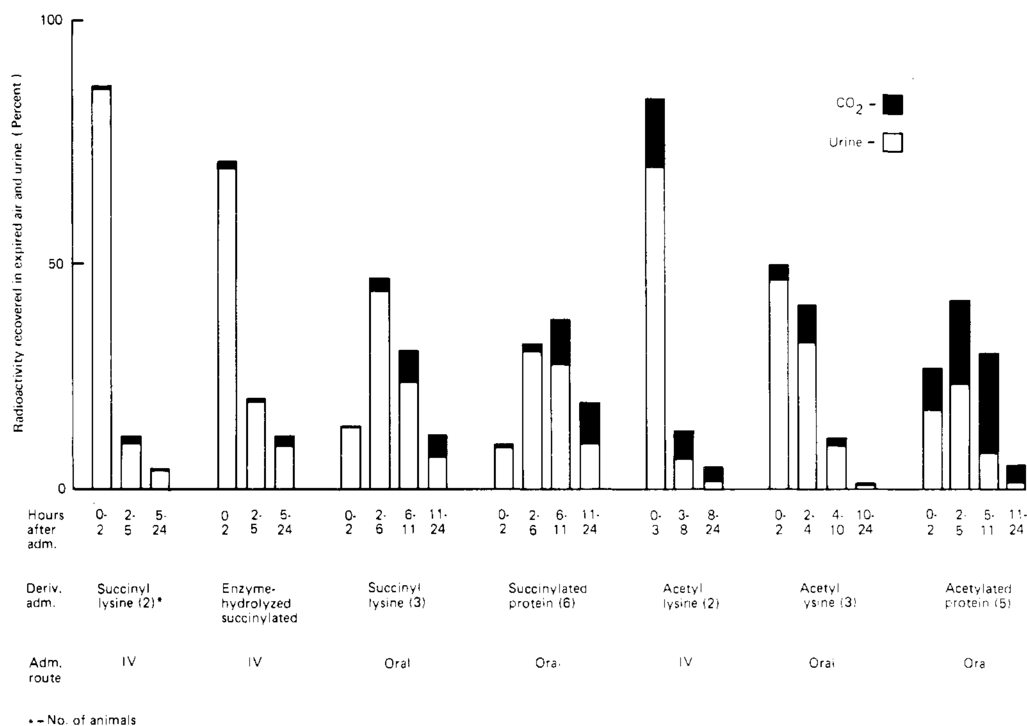

Figure 1. Recovery of radioactivity in CO_2 and urine during 24 h following the administration of ^{14}C acyl protein or lysine to rats.

greater proportion of recovered radioactivity found in the expired CO_2 from acetyl derivative treated animals. In all trials, $^{14}CO_2$ appeared sooner in acetyl derivative treated animals. Comparison of the distributions from animals given [^{14}C]acetyllysine or [^{14}C]succinyllysine intravenously indicated that nearly 25% of the acetyllysine is available, but the succinyllysine is not. Radioactivity recovery data indicated that acetylated protein was approximately 50% utilizable, but the orally administered acetyllysine was only 14% utilizable. The distribution pattern of succinyllysine, given orally, was rather similar to that of succinylated protein. Absorption and excretion of orally administered succinyl derivatives apparently required much more time than for absorption and excretion of orally administered acetyl derivatives.

The relatively large amount of $^{14}CO_2$ evolved during the latter portion of the 24-h test period could be caused by microbial flora. This was suggested by Tanaka et al. (1975) in work on the absorption of fructose-L-tryptophan and by Finot et al. (1977a) in feeding tests involving lysinoalanine. When hydrolyzed succinylated protein was administered intravenously to the rat, only very small amounts of radioactivity were recovered in $^{14}CO_2$; this result, which is very similar to that obtained when succinyllysine was used, indicates nonutilization. These results lend further weight to our suggestion that the apparent utilization of orally administered succinyllysine and protein derivatives might be due to the action of gut microorganisms.

We have no explanation for the variability in the utilization of the acetyl derivatives relative to the kind of derivative (lysine or protein) and relative to the route of administration (intravenously or oral). Also, we cannot explain the apparent discrepancy between the 14–23% utilization found for acetyllysine in this study and the reported utilization of approximately 50% for acetyllysine (Boggs, 1977; Finot et al., 1977b).

No significant amounts of radioactivity were found in the rat feces nor in that rat organs removed at the end of the 24-h period.

It can be concluded from the radioactivity recovery data that acetyllysine, both free and protein-bound, was partially hydrolyzed. Intravenously administered succinyllysine was not utilized; however, there may have been some utilization of the orally administered succinyllysine and protein derivatives. The utilization picture for both orally administered acetyl and succinyl derivatives may have been distorted somewhat by microbial metabolism of these derivatives in the rat gut.

B. Separation and Characterization of Recovered Radioactive Components from Urine. The radioactive components of urine were separated from other urine components by gel filtration and subsequently by TLC on cellulose. The radioactive components that were eluted from cellulose plates were hydrolyzed and subsequently analyzed quantitatively by amino acid analyzer.

1. Acetyllysine. When [^{14}C]acetyllysine was administered intravenously to the rat, the major radioactive component found in the urine was acetyllysine (Figure 2 and Table V). Orally administered [^{14}C]acetyllysine gave similar results; however, small amounts of serine, glycine, and glutamic acid were also found. These results are in general agreement with those of Bjarnason and Carpenter (1969b) in which they showed that acetyl- and propyllysine were apparently excreted by the rat.

The urine from the rat fed ^{14}C acetylated protein gave what appeared to be a single major radioactive component when it was fractionated by TLC; however, this component(s) had a greater R_f than acetyllysine. It gave a very light pink color after treatment with ninhydrin, and amino analysis showed it to contain about 50% as much glycine plus very small amounts of glutamic and serine. This suggests that ^{14}C-labeled acetyllysine may be combined with glycine and/or that it may be in the diacetyl form as acetylation is the classic detoxification process of amino derivatives in the kidney (Finot et al., 1977a). This work clearly shows that the excretory products from animals fed acetylated protein different from the products from animals fed acetyllysine. However, it is not known what were the exact differences between these excretion products.

2. Succinyllysine. The characteristics of the excretory products from animals administered ^{14}C-labeled succi-

Table V. Composition of Major Radioactive Urinary Components from Rats Fed Labeled Acylated Lysines and Proteins

		amino acid (μM/24 h)						
derivative	route of administr.	lysine	glycine	glutamic acid	serine	alanine	aspartic acid	proline
acetyllysine	iv	52.4	0	0	0	0	0	0
acetyllysine	oral	127.2	2.3	2.5	3.9	0	0	0
acetylated protein	oral	10.4	5.3	0.2	0.2	0	0	0
succinyllysine	iv	*a*	*a*	*a*	*a*	*a*	*a*	*a*
succinyllysine	oral	*a*	*a*	*a*	*a*	*a*	*a*	*a*
succinylated protein	oral	*a*	*a*	*a*	*a*	*a*	*a*	*a*

[a] Identified, but not quantitatively measured.

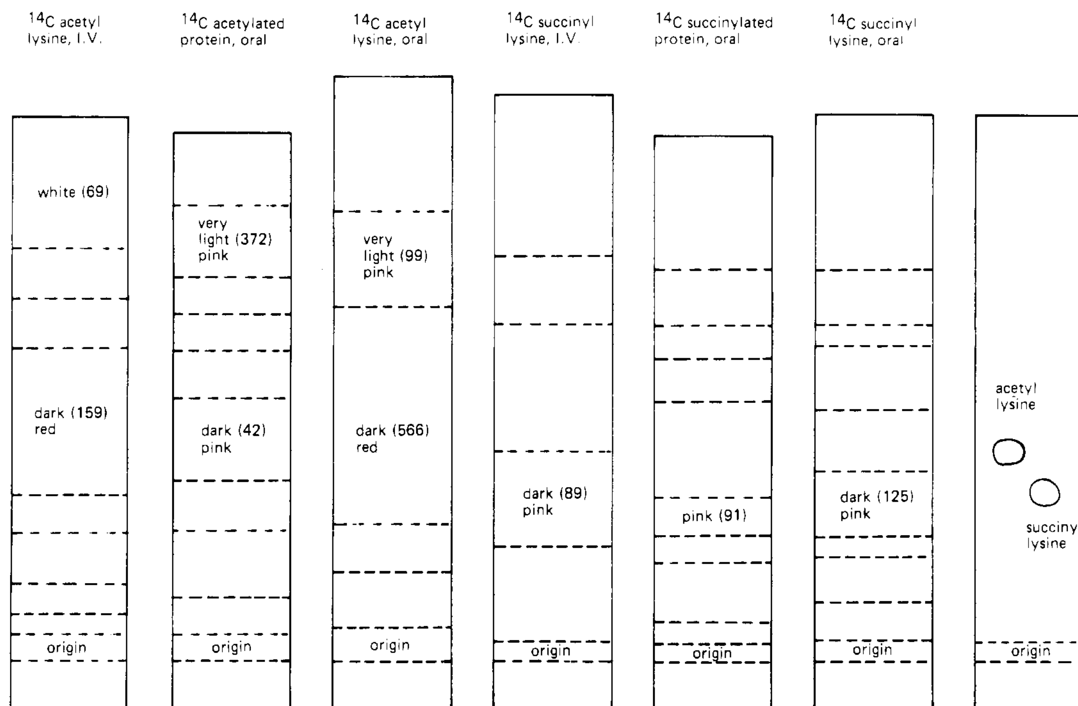

Figure 2. Thin-layer chromatograms of urines from rats administered ^{14}C acyl derivatives. Conditions given in methods. Radioactivity in major bands is given in counts per minute.

nyllysine or ^{14}C-labeled succinylated protein appeared to be similar. These products were the only significant radioactive components; their R_f values on cellulose were similar to that of succinyllysine, and these components yeilded a bright red ninhydrin derivative. After hydrolysis, these components were shown to contain lysine and other amino acids.

SUMMARY

Succinylation of fish myofibrillar protein resulted in up to 35% of the cysteine being converted into the *S*-succinyl derivative; however, acetylation resulted in about two-thirds of this amount of *S*-acetyl derivative. Highly acetylated fish myofibrillar protein had a PER of 85% that of reference casein and medium succinylated fish protein had a PER of 73% of that of reference casein; however, the rate of tryptic hydrolysis of acylated substrates was lower than that of unacylated substrates.

The feeding of ^{14}C-labeled acetyllysine and ^{14}C-labeled acetylated protein to rats showed that both free and protein-bound acetyllysine are partially utilized. ^{14}C-labeled succinyllysine and succinyllysine in an enzyme hydrolysate of ^{14}C-labeled succinylated protein, both administered intravenously, were not utilized. There may have been, however, some utilization of orally administered free and protein-bound succinyllysine. The utilization of orally administered acetyl and succinyl derivatives may be distorted somewhat due to gut microbial activity.

Fractionation of the urine from rats given ^{14}C acetylated lysine or protein showed that nonutilized [^{14}C]acetyllysine was excreted as acetyllysine. The nonutilized [^{14}C]-acetyllysine from ^{14}C acetylated protein given to rats was excreted as acyllysine but it was probably associated with glycine or perhaps it was in the diacetyl form. It appears that succinyllysine derivative may be excreted as succinyllysine.

These results show that acetyl- and succinyllysine derivatives were only partially utilized and that the level of utilization was dependent on the type of acyl group of lysine. This was reflected in lowered PER values.

Use of trade names in this publication does not imply endorsement of commercial products.

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Some Properties of Pea Lipoxygenase Isoenzymes

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Four pea lipoxygenase isoenzymes were isolated from pea seeds (Little Marvel variety) by ammonium sulfate fractionation, gel filtration, and ion-exchange chromatography. The two major isoenyzmes, pea lipoxygenase I and II (PL I and PL II), were further purified by isoelectric focusing in a granulated gel. PL I and II were homogeneous proteins and had isoelectric points of 6.25 and 5.82, respectively. The molecular weight of both PL I and II was 95 000 and they had optimum activity between pH's 5 and 7 and no activity above pH 8. The two minor isoenzymes were present at very low concentrations and were active at pH 9, but not at neutral to acid pH's. PL I and PL II differed in pH profiles, substrate specificity, carotene- and chlorophyll-bleaching activity, and in their ability to produce carbonyl compounds during the linoleate oxidation reaction.

Quality deterioration, such as off-flavors, off-odors, and off-colors, in unblanched frozen vegetables has been ascribed to the oxidative degradation of unsaturated lipids by certain naturally occurring enzymes (Joslyn, 1949; Lee and Wagenknecht, 1951, 1958). Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12), which catalyzes the peroxidation of unsaturated fatty acids, is recognized as one of the main oxidative catalysts in plants. Interest in the role of lipoxygenase in the formation of flavor and volatile substances from lipids and in the cooxidation of pigments ranges from practical use to basic questions about the assumed secondary reactions (Eskin et al., 1977).

The development of rancid off-flavor in unblanched vegetables during frozen storage was associated with increases in hydroperoxides and carbonyl compounds which were thought to be the result of lipoxygenase action on lipids in vegetables (Siddiqi and Tappel, 1956; Wagenknecht and Lee, 1958; Bengtsson and Bosund, 1966). The role of lipoxygenase in the development of beany and bitter flavors from lipids during the processing of soybeans has been demonstrated (Mustakas et al., 1969; Kon et al., 1970; Kalbrener et al., 1974). However, the routes leading to the formation of flavor and aromatic compounds and the involvement of lipoxygenase in these reactions remain unclear.

Lipoxygenase, which was named "carotene oxidase" initially, was also thought to cooxidize carotene and chlorophyll (Grossman et al., 1969; Kies et al., 1969; Holden, 1965; Arens et al., 1973). Much of the work concerning the participation of lipoxygenase in pigment bleaching was done with enzyme preparations of varying degrees of purity, but differences in bleaching potential among isoenzymes has been suggested. This indicates the importance of using purified enzymes for studying the bleaching activity of lipoxygenase (Kies et al., 1969; Grosch et al., 1976; Pistorius, 1974).

The existence of multiple lipoxygenases in soybeans is well-established (Guss et al., 1967; Yamamoto et al., 1970; Verhue and Francke, 1972; Christopher, 1972) and their occurrence in other vegetables has been indicated. The

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