haps the minor subgroups hold significance in this relationship.

Summary. Three γA myeloma proteins were encountered which were very similar but differed markedly in antigenic properties from the bulk of γA proteins. Several antisera from rabbits as well as primates served to distinguish these proteins as belonging to a well defined subgroup. The distinguishing characteristics were shown to be properties of the heavy chains. A general but not absolute relationship to electrophoretic mobility was demonstrated.

ADDENDUM. Exchange of γA proteins with Drs. Vaerman and Heremans and also with Drs. Feinstein and Franklin indicate that these two groups of workers have also observed the same subgroups.

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Sulfide Liberation from Raw Soybean Protein. (31288)

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Numerous reports have been concerned with the factors or properties that cause an inferior nutritive value of raw soybean meal as compared to properly cooked meal. These reports have been reviewed recently(1). Among such factors have been anti-enzymes, pancreatic hypertrophy, amino acid deficiency or unavailability, toxic proteins, and indigestible protein fractions, but the effects or even the existence of some are still matters of disagreement.

Proteins which are known to be highly indigestible, *i.e.*, the keratins, have been shown to be improved in digestibility by steam cooking under pressure. A comprehensive report (2) has reviewed the literature in this field and presented specific data in respect to feather protein. It was shown that the principal changes on treatment with steam were loss of cystine, corresponding appearance of lanthionine and increased susceptibility to enzymatic hydrolysis. The conversion of cystine in part to lanthionine, which involves breakage of disulfide cross links between amino acid chains and loss of one-half the sulfur, was most rapid during the first 30 minutes of processing at 30 lb steam pressure. At the same time, amino nitrogen liberated by rupture of peptide bonds increased very slightly, if at all.

Some data(3) obtained during steam processing of hoof and horn meal are in agreement with the later studies on feather protein. Volatile sulfur, particularly H_2S, was detected in the exhaust gases. Neartly two-thirds of the original cystine was lost, while the apparent digestibility to pepsin and HCl improved from an original 13 to 73%. The loss of sulfur

from proteins during cooking has been known for a long time. Recently, the cooking of cod muscle has been shown to result in a loss of cystine(4). We investigated the possibility that such changes could take place in other protein sources such as the soybean.

Methods and results. A 5 g sample of raw soybean meal, moistened with water, was placed in a flask with a narrow neck over which was fastened 2 layers of filter paper soaked in lead acetate solution. This flask was placed in a pressure cooker containing water. In the first 30 minutes of cooking at 15 lb pressure, a very distinct deposition of lead sulfide took place on the test papers. Almost none appeared on the outer paper. A further 30 minutes of cooking of the same sample liberated a relatively small amount of sulfide, indicating that the releasing process was essentially complete in the first 30 minutes of cooking. Commercial toasted soybean meal also released some sulfide, but much less than that observed with raw meal. Quantitative estimations of the H_2S evolved from raw meal showed that the volatile sulfur was approximately 2.3% of the total cystine sulfur present.

Raw soybean meal was fractionated into a water-insoluble protein fraction, and a water-soluble but isoelectrically precipitated protein fraction, by a procedure described(5). Testing these for evolution of sulfide during one hour of cooking in steam at 15 lb revealed that only the water-insoluble residue protein liberated sulfide. This showed clearly the existence of at least 2 proteins that differ markedly in sulfide lability and that the active protein remained in the insoluble residue, as might be expected of a difficultly digested protein. On basis of the distribution of protein and the estimations of sulfide found, it was calculated that 4-5% of the cystine sulfur present in the residue protein(s) could have been liberated. If this is assumed to have come from disulfide cross links converting to lanthionine, then possibly 10% of the cystine of residue protein was involved in such links.

In addition, we have determined that the sulfide-liberating component is not in the hexane or ether-extractable fraction. Samples of isolated trypsin inhibitor from soybean and from egg did not liberate sulfide on pressure cooking.

Treatment of raw meal with pepsin and HCl, as for usual pepsin digestibility measurement also liberates sulfide; however, the acid alone does not. Treatment of raw meal with trypsin at pH about 7 liberates only traces of sulfide. It is apparent that pepsin is capable of attacking the linkages which can be broken to liberate sulfide. This might mean that an animal with a strong peptic digestive system would be less affected by the difficulty of digesting a portion of the raw soybean.

These observations strongly indicate that the same type of enzyme-resistant disulfide linkages known to be present in keratin proteins can exist to some extent in soybean and probably other proteins. It is suggested, therefore, that raw soybean meal, in addition to all other varied causes for reduced protein efficiency, contains amino acid structures involving cystine which are deterrent to trypsin enzymatic rupture and which are destroyed or modified by steam cooking. This clarifies at least part of the explanation of generally poor nutritional results with raw soybean protein and agrees with the evidence already presented or reviewed(1), that raw soybean protein has a lower overall digestion coefficient as compared to cooked soybean protein.

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