Table I. Copper, Nickel, and Cobalt Reported in Sea Water

	Micrograms per Liter		
Reference	*Cu*	*Ni*	*Co*
(*2*)	<3000	1.5–6	<0.3
(*4*)	4–10	0.1–0.5	0.1
(*6*)		0.7–0.8	0.38–0.67
(*7*)		2	
(*8*)			0.23–0.32
(*10*)	20	3	1
(*11*)	13–22		0.33–0.67

other investigators. If the respective values of 18, 2, and 0.5 γ per liter are accepted for copper, nickel, and cobalt in sea water, results of the present work indicate that copper and cobalt are concentrated in the oyster shell to nearly the same extent, 1.8×10^3 and 2×10^3, respectively. The concentration of nickel, however, from sea water to shell is appreciably higher at 1.5×10^4.

Copper in the edible portion of oysters has been reported by various workers to vary over the wide range of about 5 to 2000 p.p.m. in the living matter. A fair average of the compiled data of Vinogradov for *Crassostrea virginica* would be approximately 1100 p.p.m. of copper. As the edible portion of oysters contains about 87% water, it is evident that on the dry basis this part, even for the lower ranges of copper recorded in the literature, contains more of this element than found in the shell in the present study. The single reference to copper in oyster shells which was noted (*9*) gave a value of 25 p.p.m., which is close to the findings reported here.

Figures quoted by Vinogradov on nickel in gills, mantle, and hepatopancreas of *Crassostrea angulata* are 1.3, 0.9, and 1.0 p.p.m., respectively, and a value for nickel in the edible portion of *Crassostrea sp.* is given as 1.7 p.p.m. No reported reference to nickel in the shell was found. The author's value of 30 p.p.m. indicates that, unlike copper, there is considerably more nickel in the shell of oysters than in the edible portion.

Cobalt does not appear to have been determined in either edible portion or shell of oysters, though the element has been recorded in living matter and various organs of a few genera of Mollusca (*10*). The limited data indicate that, in general, cobalt is present in smaller quantity than nickel and that the nickel-to-cobalt ratio is about the same as that found in sea water.

Although the cobalt content of oyster shells is higher than that of most plant products, it is far too low to serve as a source of this element in cobalt-deficient areas by the application of lime derived from oyster shells. In regions of the world where maintenance of health in ruminants requires the addition of a cobalt supplement to fertilizer or lime, 2 pounds of the commercial sulfate per acre will last for 3 to 5 years. In other words, about 0.1 pound of cobalt per year will be needed, whereas the application of even 5 tons per acre of oyster shells would furnish only 0.01 pound of cobalt.

The copper content of oyster shells is probably too low for it to exert any significant effect in correcting a deficiency of this element in plants and soils. In various soils throughout the world where copper deficiencies for livestock, citrus fruits, or other crops occur, an application of commercial sulfate varying from 5 to 50 pounds per acre has been employed, and the effect usually lasts at least 3 years. A minimum of about 1 pound of copper per acre per year is thus indicated, whereas even 5 tons per acre of oyster shells would furnish only about 0.3 pound of copper.

There have been several reports on the beneficial effect from cobalt additions to poultry rations (*1*, *3*). From 1 to 12 p.p.m. of cobalt in the feed have resulted in increased growth. It is possible, therefore, that the minute quantities of cobalt in oyster shells may have significance in poultry nutrition.

Literature Cited

(1) Berzins, J., Rozenbachs, J., *Latvijas PSR Zinātņu Akad. Vēstis* **1953,** No. 9, 39–46.

(2) Black, W. A. P., Mitchell, R. L., *J. Marine Biol. Assoc. United Kingdom* **30,** 575–84 (1952).

(3) Burns, M. J., Salmon, W. D., J. Agr. Food Chem. **4,** 257–9 (1956).

(4) Harvey, H. W., "Recent Advances in the Chemistry and Biology of Sea Water," Cambridge Univ. Press, Cambridge, Eng., 1945.

(5) Hopkin and Williams, Ltd , Chadwell Heath, Eng., "Organic Reagents for Metals," 5th ed., p. 175, 1955.

(6) Ishibashi, M., *Records Oceanog. Works Japan* [N.S.] **1,** No. 1, 88–92 (1953).

(7) Laevastu, T., Thompson, T. G., *J. conseil, Conseil permanent intern. exploration mer* **21,** 125–43 (1956).

(8) Thompson, T. G., Laevastu, T., "Determination and Occurrence of Cobalt in Sea Water," Alaskan Science Conference, 1956.

(9) Tressler, D. K., "Marine Products of Commerce," p. 529, Chem. Catalog Co., New York, 1923.

(10) Vinogradov, A. P., "The Elementary Chemical Composition of Marine Organisms," pp. 341, 352, 364, Sears Foundation for Marine Research, New Haven, 1953.

(11) Young, E. G., Smith, D. G., Langille, W. M., *J. Fisheries Research Board Can.* **16,** 7–12 (1959).

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DIETARY PROTEIN VALUES

Complete *vs.* Total Protein in the Evaluation of Diets

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The protein content of foods is customarily determined by multiplying the nitrogen content of the food by one of several factors which range in magnitude from 5.7 to 6.66. The misleading impression that such factors may impart concerning the real protein content of mixed foods has been discussed (*5*). However, even if the protein content is determined by more adequate means, the result may or may not describe the value of this protein for nutritive purposes. The concept of "complete protein" was introduced (*7*) to define more clearly the quantity of protein which would be available to the organism for repair and synthesis of tissue. Complete protein is, by this definition, that fraction of the total dietary protein which is completely utilized for anabolic purposes. As the pattern of essential amino acids required by an animal appears to depend on its physiological state (growth, tissue repair, pregnancy, lactation, adult maintenance), the amino acid composition of the complete protein will vary with the animal's requirements. In this study, complete protein is represented by the pattern of essential amino acids needed

Determination of the total protein content of food is not an adequate index of its nutritive value. Experiments in which rats were fed diets containing approximately equal amounts of total (crude) protein but differing from each other in their content of several essential amino acids and in their content of complete protein, show that the nutritive value of a food as a source of protein is closely correlated with its content of complete protein.

for growth of the young rat. The complete protein content of a food or diet is calculated from the equation:

$$P_c = Q \times \frac{A}{Ar}$$

where

P_c = percentage of complete protein in food

Q = percentage of total protein N × 6.25

A = quantity of limiting amino acid, grams per 16.0 grams N

Ar = requirement for limiting amino acid, grams per 16.0 grams N

The complete protein content cannot, by definition, exceed the total protein of the diet.

The experiments described here show that when various mixtures of white flour, lactalbumin, and lysine are fed to rats at the same level of total protein (N × 6.25), there is no relationship between the growth rate and the total protein (N × 6.25) content of the diets. On the other hand, when the complete protein content of the diets is used as the criterion, then, in accord with previous experiments (*7*), the growth response, the food consumption, etc., are significantly related to the amount of complete protein in the diets.

Table I. Growth Response of Rats on Diets of Equal Total Protein but Different Complete Protein Content

Supplement per 100 G. Wheat Flour		*Protein*		*Weight Gain*		
Lactalbumin, g.	*Lysine. HCl, mg.*	*Total, %*	*Complete,[a] %*	*4-week, g.*	*Av. daily, %*	*Limiting Amino Acid Assumed*
33	0	15.2	15.2	121	4.09	Cys-Met
25	0	15.3	15.3	130	4.21	Cys-Met
19	0	15.2	14.9	134	4.25	Cys-Met
14	0	15.1	14.0	129	4.18	Cys-Met
11	0	15.2	13.5	121	4.09	Cys-Met
8	0	15.2	12.6	110	3.92	Lysine
0	580[b]	15.1	11.6	82	3.39	Cys-Met
0	450[b]	15.0	11.6	96	3.67	Cys-Met and Lysine
0	710[b]	15.1	11.5	91	3.59	Cys-Met
0	760[b]	15.0	11.3	88	3.65	Cys-Met
0	830[b]	15.0	11.3	91	3.59	Cys-Met
5	0	14.9	10.4	85	3.45	Lysine
0	330[b]	15.1	10.3	93	3.57	Lysine
0	450	14.9	9.8	76	3.25	Cys-Met
0	330	14.9	9.8	76	3.25	Cys-Met
3	0	15.1	9.1	65	2.97	Lysine
0	220[b]	15.1	8.9	72	3.17	Lysine
0	220	15.1	8.9	67	3.07	Lysine
1.3	0	15.1	7.6	38	2.11	Lysine
0	100	15.0	7.5	40	2.18	Lysine

[a] Complete protein calculated on basis of limiting amino acid shown in column 7.

[b] Also contained 100 mg. of DL-methionine, 50 mg. of DL-threonine (allo-free), and 40 mg. of DL-tryptophan.

Experimental

Diets. Mixtures of high protein wheat flour (N = 2.67%) and lactalbumin (N = 12.8%) or of wheat flour and lysine.HCl (95% L-lysine.HCl) were added to starch, corn oil, minerals, and vitamins, so that all the final diets contained 15% total protein (N × 6.25). Details of the preparation of the diets have been described (*7*).

Amino Acids. The amino acid compositions of the diets were calculated from the composition of wheat flour (*5, 8*) and lactalbumin (*6*) and confirmed, in part, by analyzing the diets for lysine by paper chromatography (*6*).

Feeding. One hundred and twenty weanling male rats, weighing 45 to 50 grams each, were randomly distributed among 20 groups of six rats each. The animals were housed in individual cages and each animal received, *ad libitum*, water and one of the experimental diets. Individual records were kept of the weekly weight gains and the food consumption for the 28 days of the experiment.

Results and Discussion

Table I shows the total and complete protein contents of the diets and the weight gains. The dietary level of complete protein was calculated using the equation given above. In this calculation it was assumed that complete protein contains 5.3 grams of lysine, 4.6 grams of valine, 5.0 grams of sulfur amino acids (cystine plus methionine), 3.0 grams of threonine, and 1.1 grams of tryptophan per 16 grams of nitrogen (*1, 2*), although the following pattern was suggested by one of us (*4*) in 1945: arginine 4.7, histidine 2.0, lysine 5.2, tyrosine plus phenylalanine 8.6, tryptophan 1.1, cystine plus methionine 4.1, and threonine 3.6. The evidence that complete protein contains approximately 5.3 grams of lysine per 16 grams of nitrogen is considerable (*7*); however, the values for the remaining essential amino acids are less well documented.

In agreement with Bender's findings (*3*), the maximum growth rate attained on wheat flour supplemented with lysine does not exceed that corresponding to 65 to 70% complete protein. If the assumed estimate of amino acid requirements is correct, the second limiting essential amino acid in flour should be cystine plus methionine. However, experiments reported in the literature (*3, 11*) suggest that threonine is the second limiting amino acid in wheat proteins. This discrepancy suggests that the estimated requirements for the sulfur amino acids are too high and those for threonine too low. There is considerable evidence that the threonine requirement is in the order of 3.0 to 3.8 grams per 16.0 grams of nitrogen (*4, 5*) when amino acid mixtures simulating good animal proteins are used in the test ration. If the requirements for threonine are higher when wheat proteins are the source of the amino acids, the possibility of an amino acid imbalance causing this increased need should be considered. However, the levels of the sulfur amino acids, and of threonine, valine, and tryptophan in wheat proteins, are such that an error of 10 to 20% in the estimated requirements for any of these would change the identity of the second and third limiting essential amino acid without appreciably affecting the calculated quantity of complete protein.

In spite of these possible sources of error, statistical analysis of the results reveals that there is a significant relationship (correlation coefficient, r = 0.93) between the calculated amount of complete protein in the diet and the growth response (Figure 1) and that

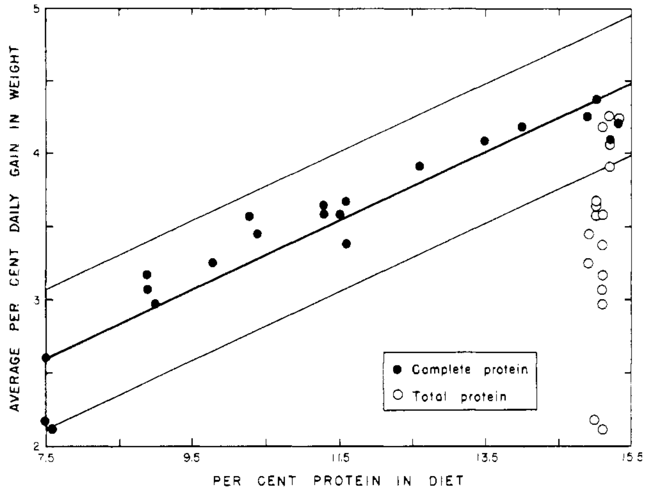

Figure 1. Correlation of average daily weight gain with complete and total protein

Fine lines represent range within which 95% of values may be expected to fall

there is a lack of correlation between the total protein in the diet and the growth response.

The results described in this paper demonstrate again that the nutritive value of foods is not adequately expressed by their total protein content (N × a factor) and that a better, although certainly not perfect, method would be the evaluation of foods on the basis of their complete protein content. This method of protein evaluation is not something for the distant future, but can be used with reasonable confidence at present. The amino acid composition of the important food proteins (*6, 8, 10*) and the amino acid pattern of complete protein (*2, 4*) are already known with sufficient accuracy to be used in many practical applications. One further point is that the amino acids in the food which are being evaluated must be available to the organism. If one or more essential amino acids have been rendered partly unavailable, because of underheating in the case of many legumes or by overheating (toasting) in the case of certain cereals, the amount of this loss must be taken into account and the quantity of complete protein must be corrected accordingly.

The concept of complete protein is also useful in another respect. For example, certain methods of processing milk result in the biological loss of approximately 10% of its lysine (*9*). This would be of importance when the milk proteins are employed to supply lysine to lysine-deficient diets. However, in diets in which lysine is present in relative abundance but total protein is limiting, the inactivation of 10% or so of the lysine is immaterial. Milk proteins are approximately 80% complete, as they contain 4 grams of cystine plus methionine per 16 grams of nitrogen and the assumed requirement is 5 grams. As milk contains 8.0 grams of lysine per 16 grams of nitrogen (requirement is 5.3 grams), almost one half of the lysine in milk could be lost during processing without diminishing the amount of complete protein. Such an extensive loss of lysine is never caused by modern methods of processing milk.

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Literature Cited

(1) Allison, J. B., *J. Am. Med. Assoc.* **164,** 283–9 (1957).
(2) Allison, J. B., *Voeding* **19,** 119–38 (1958).
(3) Bender, A. E., *Science* **127,** 874–5 (1958).
(4) Block, R. J., Bolling, D., "Amino Acid Composition of Proteins and Foods," Charles C Thomas, Springfield, Ill., 1945.
(5) Block, R. J., Mandl, R. H., *J. Am. Dietet. Assoc.* **34,** 724–6 (1958).
(6) Block, R. J., Weiss, K. W., "Amino Acid Handbook. Methods and Results of Protein Analysis," Charles C Thomas, Springfield, Ill., 1956.
(7) Howard, H. W., Monson, W. J., Bauer, C. D., Block, R. J., *J. Nutrition* **64,** 151–65 (1958).
(8) Kuppuswamy, S., Srinivasan, M., Subrahmanyan, V., "Proteins in Foods," Indian Council of Medical Research, New Delhi, India, 1958.
(9) Mauron, J., Mottu, F., *Arch. Biochem. Biophys.* **77,** 312–27 (1958).
(10) Orr, M. L., Watt, B. K., "Amino Acid Content of Foods," U. S. Government Printing Office, Home Economics Research Rept. **4** (1957).
(11) Sure, B., J. AGR. FOOD CHEM. **5,** 373–5 (1957).

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REVIEW OF FILLED MILK

Nutritional Evaluation of the Replacement of the Fat in Whole Cow's Milk by Coconut Oil

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THERE IS GROWING CONCERN among nutritionists and others regarding the far-reaching implications of the use of additives and substitutes in industrially prepared foods. In various milk-deficit areas of the world coconut oil mixed with nonfat milk solids is offered as a replacement for whole milk, for all purposes, including the feeding of infants.

It is desirable to know to what extent nutritional intakes are jeopardized by this substitution, especially in the feeding of children who in some regions, may be already on suboptimal diets. The purpose of this study is to review the findings and facts that bear directly or indirectly on the comparative nutritional qualities of milk fat and coconut oil, and the implications of the substitution of the one for the other in whole cow's milk.

Fats other than coconut oil do not appear to be extensively used as a substitute for milk fat in the production of filled milks, except in the United States. Coconut oil is the cheapest of the usable oils in most tropical countries. WHO reports that some olive oil is used in Spain and peanut oil in El Salvador. Hydrogenated cottonseed oil appears to be the substitute fat generally employed in the United States.