tial change propagates through the A-V node. The slow potential, in turn, depolarizes the bundle.

It is apparent from the differences in potential shapes at various nodal sites, and from changes during first degree block, that the slow potential is propagated. Velocity, calculated from measurements of the time of activation of atrial cells in the nodal region, nodal length, and time of bundle activation, range from 0.12 to 0.04 m/sec. It is possible that the lack of rapid deflections from the A-V node results from a difference in the nature of the cellular activity—that is, A-V nodal depolarization involves a slower voltage change than depolarization of other cardiac cells. Study of the buried nodal cells with intracellular electrodes should answer this question, but preliminary experiments have thus far been unsuccessful. Comparison of the slow potential to the end-plate potential seems inappropriate, since (i) retrograde ventriculoatrial excitation is possible; (ii) chemical transmission is probably not involved, as it is in end-plate transmission (*9*); and (iii) the slow potential is propagated.

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16 December 1957

Nutritive Value of Bread Protein Fortified with Amino Acids

Considerable interest has been aroused in the fortification of foods with amino acids and particularly, in view of its quantitative importance, in the fortification of bread with lysine. Several authorities have claimed that large increases in the nutritional value of bread protein result from such fortification, but unfortunately these claims are gross exaggerations.

Table 1. Net protein utilization (NPU) of bread fortified with amino acids.

Item	No. of assays	NPU	± S.E.*
Bread	4	46	± 0.9
Bread + L-lysine (0.2%)	3	57	± 0.9
Bread + L-lysine (0.2%) and DL-threonine (0.88%)	4	70	± 3.2
Bread + L-lysine (0.28%), DL-threonine (1.4%), and DL-methionine (1.1%)	7	79	± 1.4

* S.E., standard error.

The error seems to have arisen from a confusion of the two terms *protein efficiency ratio* (gain in weight per gram of protein eaten) and *biological value* (the percentage of absorbed protein retained by the experimental animal).

Rosenberg and Rohdenburg (*1*) showed that the protein efficiency ratio (PER) of bread protein can be increased from 1.01 to between 1.89 and 2.12 by fortification with lysine. Several authors [Jolliffe (*2, 3*); Flodin (*4*); Horder, Dodds, and Moran (*5*); and Frazer (*6*)] have misinterpreted this finding as an approximate doubling in the biological value of the protein and even that lysine-fortified bread approaches a biological value of 100. The calculation of Flodin (*7*) that the addition of lysine would add to the individual's diet the equivalent of 27 lb of meat, or 70 qt of milk or 330 eggs is based on the same error.

Protein efficiency ratio is a somewhat imprecise method of measuring nutritive value as it varies with food intake (*8, 9*). Moreover, a protein efficiency ratio of zero is recorded with protein of which 30 to 40 percent is retained by the animal (*9, 10*). Biological value or net protein utilization (biological value × digestibility) is independent of food intake and ranges from 0 to 100. Therefore the nutritive value of bread protein that had been fortified with various amino acids was investigated by measuring the net protein utilization (NPU) by the carcass water analysis method of Bender and Miller (*11*).

Sliced loaves were purchased from a grocery shop at a time (1955) when the National Loaf was nominally 80 percent extraction although, in fact, it was considerably lower than this (*12*). The slices were dried in air at 50°C, crumbed, and powdered in a hammer mill. The diet contained 70 percent bread, 15 percent margarine fat, 5 percent potato starch, 5 percent glucose, and an adequate supply of minerals and vitamins (*11*). The amino acid-fortified diets contained the following additives, (i) L-lysine monohydrochloride (0.2 percent lysine); (ii) L-lysine + DL-threonine (0.88 percent); and (iii) L-lysine (0.28 percent) + DL-threonine (1.4 percent) + DL-methionine (1.1 percent). Diets were fed at 1.5 percent nitrogen level for the 10-day experimental period. The results are presented in Table 1.

These results show that, in bread, threonine is the second limiting amino acid and that methionine (or methionine and cystine) is the third. Sure reported (*13*) that threonine was the second limiting amino acid and valine the third in milled wheat flour, and that in whole wheat (*14*) valine was the second and threonine the third. Whether bread, whole wheat, and milled wheat flour do differ in their second and third limiting amino acid is not clearly established.

Rosenberg, Rohdenburg, and Baldini (*15*) concluded that bread containing skim milk solids lacked only lysine. This conclusion was based on two observations: (i) the bread + lysine diet permitted growth equal to that on stock diet, (ii) further supplementation with valine, threonine, and methionine produced no improvement in protein efficiency ratio. The contrary findings in the present paper are not due to the different types of bread used in Great Britain and the United States but to some inadequacy in the diets used by Rosenberg *et al.* who obtained maximum protein efficiency ratios of only 1.8 to 2.4 (corresponding to biological values of 60 to 70) whereas the best protein reaches a protein efficiency of about 4.4 (*9*).

The results shown in Table 1 place in true perspective the increase in nutritional value conferred by the addition of lysine. They agree remarkably well with the values obtained by the method of protein efficiency ratio by Rosenberg and Rohdenburg (*1*) and Hutchinson, Moran, and Pace (*16, 17*). The protein efficiency ratios found by these authors have been converted into the approximate equivalent of net protein utilization in Table 2 by use of the conversion factor of Block and Mitchell (*10*) and Bender (*9*).

Thus it is clear that all the experimenters are agreed that only about half

Table 2. Comparison of protein evaluations [protein efficiency ratio (PER) and net protein utilization (NPU)] of bread fortified with lysine.

Author	White bread		Bread + lysine	
	PER	Calc. NPU	PER	Calc. NPU
Rosenberg & Rohdenburg (*1*)	1.0	52	1.89	64
Hutchinson, Moran, & Pace (*16*)	1.4	58	2.14	67
Hutchinson, Moran, & Pace (*16*)	0.9	51	1.42	58
Hutchinson, Moran, & Pace (*17*)	1.25	46	2.20	68
Bender (this report)		46		57

of the protein of bread is available for the growing rat and that fortification with lysine increases this to about 60 percent. Flodin's statement (*4*), "there is a latent store of protein in bread and flour that needs only one key to release it for the body's use. That key is lysine fortification" should be amended to at least three keys, lysine, threonine, and methionine.

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5 December 1957

Zinc Requirement of the Chick

Over 20 years ago it was found that the growth of young rats was greatly impaired if their diet was extremely low in zinc. Keratinization of the skin, parakeratosis of the esophagus, loss of hair, and decreased activity of intestinal phosphatase, blood phosphatase, and carbonic anhydrase were later associated with zinc deficiency in the rat (*1*). Zinc has also been shown to be required by the mouse (*2*) and pig (*3*).

Recently, O'Dell and Savage (*4*) reported that the growth of chicks was stimulated if zinc was added to a semipurified diet containing 25 percent of Drackett assay protein C-1. No response to zinc was obtained if casein or alpha protein replaced the Drackett protein or if the level of potassium in the ration was low (*5*). All rations contained about 50 ppm of zinc. The chicks were brooded in batteries which ordinarily are zinc coated, and tap water was supplied, presumably in the galvanized water troughs of the batteries.

We have also studied this problem with chicks (*6*) but have attempted to reduce substantially the amount of zinc ingested. This was done in several ways: (i) selecting ingredients low in zinc; (ii) extracting the zinc from the protein supplement (the source of much of the zinc of the basal ration chosen); (iii) coating the batteries or building nonmetallic pens to prevent contamination with this element; (iv) conducting experiments in isolated quarters to reduce dust as a possible source of zinc; (v) using distilled water instead of tap water; and (vi) providing the drinking water in glass rather than galvanized troughs. All these changes were intended to reduce the amount of zinc ingested by the birds.

In an exploratory experiment, two groups of 5-day-old, White Leghorn cockerels were allocated to pens having plastic mesh sides and rubber mesh floors. The basal diet, similar to that used by Morrison *et al.* (*7*) without the zinc supplement, was fed to one group. This basal diet contained about 16 ppm of zinc. The second group was supplied the basal ration supplemented with 100 ppm of zinc as the sulfate, added by way of the salt premix. The chicks were allowed to eat *ad libitum* in glass feeders, and distilled water was supplied in Pyrex glass waterers.

The group which received the low-zinc, basal diet grew poorly, developed dermatitis of the feet, failed to feather properly, and developed a goose-stepping walk. Symptoms began to appear about the 14th day and were severe by the 21st day. The group fed the zinc-supplemented ration grew satisfactorily during the 4-week test period and did not exhibit any of the symptoms noted in the zinc-deficient group.

On the basis of the results of this preliminary study, a more comprehensive experiment was designed. In this, the Drackett protein was extracted by washing with a hydrochloric acid solution at *p*H 4.6 and then dried about 36 hours in an oven at temperatures up to 100°C. The unextracted basal ration contained 19 ppm of zinc; the extracted basal ration analyzed 7 ppm (*8*). Basal rations containing either the unextracted or extracted protein were fed by themselves and with 100 ppm of zinc as zinc sulfate. A total of 120 White Meteor × White Rock 1-day-old chicks were allotted on the basis of weight into the four groups, each of which contained three replicates of five females and five males each.

The experimental birds were brooded in an electrically heated battery with raised, wire floors. All battery parts were coated with epoxy plastic; the bottoms of the dropping pans and wire floors were coated with shellac. Glass founts having a plastic base supplied distilled water for drinking. The battery was isolated in a room relatively free from dust.

The basal diet was essentially that used by Morrison *et al.* (*7*) without the zinc supplement. Drackett protein was used in basal diet 1; the extracted and dried product was used in basal diet 2. Minerals were reagent-grade chemicals. The experimental diets were mixed in plastic equipment and stored in plastic bags. Experimental diets and distilled water were supplied *ad libitum* throughout the experimental period. Feed consumption was recorded.

Table 1. The effect of zinc deficiency and supplemental zinc on chick weights and feed conversion.

Group	Treatment (diet)	Av. wts* at 4 wk (g)	Gain in wt per lb of feed (lb)
1	Basal 1	183	0.45
2	Basal 1 + 100 ppm zinc	363	0.69
3	Basal 2	101	0.35
4	Basal 2 + 100 ppm zinc	333	0.62

* Significantly different. Orthogonal comparison (*9*). $P < .01$ for groups 1 and 3 versus 2 and 4; group 1 versus group 3; $P < .05$ for group 2 versus group 4.

As shown in Table 1, the birds in groups 1 and 3 grew poorly and utilized their feed inefficiently. As in the previous test, they developed a severe dermatitis of the feet, frizzled wing feathers, infantile body feathers, and a goose-stepping walk.

Addition of 100 ppm of zinc to the ration containing the unextracted protein (group 2) produced normal growth, feathering, and feed utilization. When zinc was added to the ration containing the extracted protein (group 4), response was not quite as good as it had been on the unextracted protein. This difference in performance between groups 2 and 4 shows that some factor(s) in addition to zinc was removed by extraction or that the process of extraction and drying altered the nutritive value of the protein supplement.

The results of these experiments indicate that zinc is required by the chick for growth, feather development, and maintenance of a healthy condition of the skin of the feet as well as for the efficient utilization of feed.

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