from other forms of aggregation that also involve sulfhydryl groups. Hughes (*10*) has described the formation of a mercury dimer of serum albumin, and Madsen, Cori, and Gurd (*11*) have shown an apparently new type of sulfhydryl aggregation of phosphorylase that is prevented by p-chloromercuribenzoate.

Huggins *et al.* (*12*) isolated serum albumin from normal individuals and from patients with malignant disease and found the sulfhydryl content to be the same in each group despite the fact that gel formation of plasma proteins in the latter group was abnormal. It does not appear probable that the plasma proteins from the leukemic patients have an abnormal sulfhydryl content since the ethanol precipitation phenomenon appears to depend upon the plasma nonprotein sulfhydryl level.

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"Slow" Potential Change in the Atrioventricular Node

Previous considerations of atrioventricular (A-V) conduction have been based on indirect physiological (*1*), clinical (*2*), and pharmacological (*3*) studies, but there has been no direct knowledge of intranodal events. An earlier report from this laboratory indicated the feasibility of recording from the buried portions of the A-V conduction tissue *in situ* (*4*). This technique has furnished some direct evidence about events in the A-V node (*5*).

As a small electrode is moved from the ventricle toward the atrium in the right and left bundles and then into the common bundle, the potential difference between this electrode and a distant point is symmetrical and biphasic, as would be expected in a "cable" of Purkinje fibers (*4*); this activity approaches closer in time to atrial depolarization as the electrode moves nearer to the atrium. The wave consists of a positive deflection (approaching activity), a rapid negative-going deflection (depolarization of cells near the electrode), and a terminal negative portion (receding activity) and appears earliest 30 to 40 msec after the firing of the atrial cells in the A-V nodal region. This discharge precedes the end of the P wave by about 15 msec in the dog. Similar deflections, indicating rapid depolarization of cardiac cells, were never recorded within this 30- to 40-msec interval. There thus existed a "silent" period during which no cellular firing was noted. One report of rapid potentials from the A-V node (*6*) does not place them in this silent period.

In some experiments, a slow potential change was recorded from electrodes in the A-V node during this "silent" period. When studied with direct-coupled amplifiers capable of working close to the theoretical noise limit, this change was found in the A-V node in 16 dogs.

Although many experiments were conducted on the heart *in situ*, examination of this potential required perfusion of the isolated heart, cutting of both conducting bundles, variable frequency stimulation of the sinoatrial node to produce first-degree A-V block, and exact histological localization of each electrode (Fig. 1, I). The apparatus has been described (*7*).

Potentials on an electrode in the mid A-V node are shown in Fig. 1, II. Following a stimulus to the sinoatrial nodal region (*S*), there is a large negative potential, the rapid deflection produced by depolarization of atrial cells in the A-V nodal region. Notches on the upstroke of this potential possibly result from firing of scattered cells in the atrionodal junction. The slow potential follows. It is initially positive, rising above the base line for about 20 msec, and then returns toward the base line. Fig. 1, II, shows changes that occur on increasing the rate of stimulation. The slow potential becomes prolonged, with a longer positive plateau and a long terminal negative phase.

One millimeter downstream (Fig. 1, III *c*), the slow potential has no negative phase; however, the negative-going phase coincides approximately with firing of the common bundle, 1 mm away (Fig. 1, III *b*). The slow potential varies in shape and magnitude, depending on electrode position.

In all studies, the slow potential has prolonged as the atrial stimulation rate has been raised from 2 to 3 per second to 10 per second. Concurrently, the interval between the atrial potential and the common bundle potential increases (Fig. 1, III, IV). At rates near 8 per second, the slow potential exhibits maximum prolongation, and complete (2 to 1) block occurs. During A-V rhythm (Fig. 1, IV), the slow potential precedes all other potentials and usually has a configuration differing from that during sinus rhythm.

The most likely picture of A-V nodal conduction which emerges from our study is the following: Atrial cells in A-V nodal region trigger atrionodal cells. These differ in anatomy from the atrial and the nodal cells (*8*). These cells activate the nodal cells, and the slow poten-

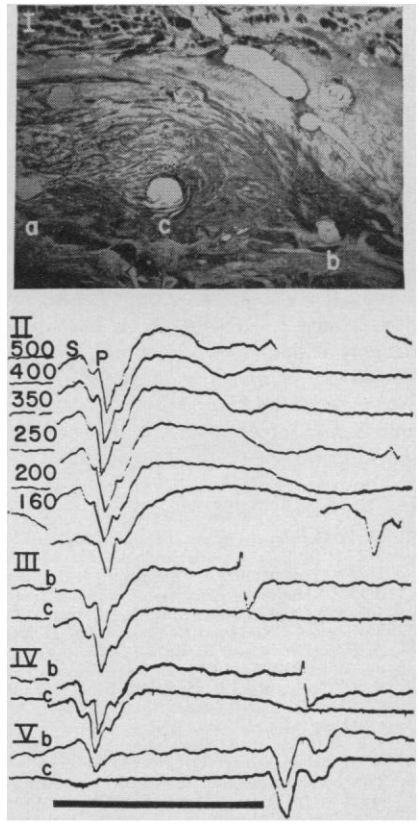

Fig. 1. (I) Histological section through the A-V node, showing locations of recording electrodes in *a*, upper A-V node; *b*, near origin of common bundle; and *c*, intranodal point midway between these. (II) Potentials recorded at point *a*; intervals between stimuli: 500, 400, 320, 250, 200, and 160 msec; *S*, stimulus artifact; *P*, auricular depolarization. At 160 msec, the common bundle did not follow the A-V node and the shape of the atrial potential was altered. The 500-msec record shows ventricular depolarization at the right. Right and left bundles had been cut, and the ventricles beat independently of the atria. (III) Potentials recorded at points *b* and *c* with interstimulus period of 400 msec. (IV) Potentials at *b* and *c* with interstimulus period of 200 msec. (V) Potentials recorded at *b* and *c* during A-V rhythm. (Parts of the auricular complex have been retouched to make the figure clearer.)

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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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