traces in Fig. 2, c and d. Thus the first peak of the magnetic trace indicates the forward loop of the currents in the moving action potential. By Ampere's law, the toroidal coil measures that part of the axial nerve current which is not canceled by return flow within the toroid. The current paths must be known in detail to determine precisely what fraction of the total nerve current is measured.

Further checks of the magnetic signals were performed. When the coils were tied to ground, rather than left floating electrically, much larger electrical artifacts were produced. Removal of the saline, so that only a slight amount of current could circulate around the still moist surface of the toroid, resulted in large, immediate signal reductions. Also, an artificial electric current dipole was made by applying a square voltage pulse to a twisted pair of No. 34 wires with bared, separated ends immersed in the saline. When this source threaded the toroid, a large signal was received; when it was oriented so that substantial field cancellation was expected, the signal dropped by a factor of 5 although the saline surrounding the toroid underwent an identical voltage excursion.

The toroid sensitivity was calculated and measured as a function of frequency (f) (80 Hz $< f <$ 10 kHz). Since the coil has resistance R in addition to inductance L, the true magnetic field waveform $B(t)$ has an additional small component proportional to the integral of the observed signal $B'(t)$ multiplied by R/L for the toroid (*10*). Thus

$$B(t) = B'(t) + R/L \int B'(t')\, dt' \quad (2)$$

with a calibration of 150 pT/mV. While the exact calculation of this correction requires inclusion of the effect of the low-pass filter (*10*), the R/L effect is small during the early part of the action potential. To show this, we approximate the first magnetic peak in Fig. 2d by a Lorentzian profile of height h = 4.8 mV and width w = 89 μsec. Then Eq. 2 becomes

$$B(0) = B'(0) + 0.30\, h/w \tan^{-1}(500/89) \quad (3)$$

resulting in a field of 125 pT at the first peak (t = 0) of which 27 percent is due to the second, correction term. This represents a net current through the toroid of 0.8 ± 0.2 μA in the forward loop of the action potential, with a precision adequate for the exploratory purposes of this work. Larger currents and larger fields will, of course, exist closer to the nerve fibers.

Even at their semiqualitative level, these experiments demonstrate that magnetic measurements of nerve function can be made directly in the conducting fluid; unlike electrical signals, they are not obliterated by the high conductivity fluid. The use of a split toroid will allow measurements on living systems without requiring puncture or intrusive contact with the nerve axon. More important, this technique measures current density directly (*14*) and allows determination of current profiles without assumptions about conductivity and electric boundary conditions that are necessary to unfold the nerve current from voltage recordings (*15*). Since the magnetic trace is very close to an actual current measurement, it is therefore a particularly strong complement to the electrical record.

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References and Notes

1. A. C. Scott, *Rev. Mod. Phys.* **47**, 487 (1975).
2. B. Katz, *Nerve, Muscle and Synapse* (McGraw-Hill, New York, 1966).
3. K. R. Swinney and J. P. Wikswo, Jr., *Biophys. J.*, in press.
4. M. Reite and J. Zimmerman, *Annu. Rev. Biophys. Bioeng.* **7**, 167 (1978).
5. J. H. Seipel and R. D. Morrow, *J. Wash. Acad. Sci.* **50** (No. 6), 1 (1960); J. A. Gengerelli, N. J. Holter, W. R. Glassock, *J. Psychol.* **57**, 201 (1964); *ibid.* **52**, 317 (1961).
6. A. Kolin, *Phys. Today* **21** (No. 5), 39 (1968).
7. A. M. Cook and F. M. Long, in *The Nervous System and Electric Currents*, N. L. Wulfsohn and A. Sances, Eds. (Plenum, New York, 1970), pp. 9–13.
8. SHE model BMP-55; S.H.E. Corp., 4174 Sorrento Valley Blvd., San Diego, Calif. 92121.
9. J. P. Wikswo, Jr., *AIP Conf. Proc.* **44**, 145 (1978).
10. J. P. Barach *et al.*, in preparation.
11. R. Lorente de Nó, *Studies Rockefeller Inst. Med. Res.* **132**, 384 (1947).
12. J. Erlanger and H. S. Glasser, *Electrical Signs of Nervous Activity* (Univ. of Pennsylvania Press, Philadelphia, 1937).
13. J. Clark and R. Plonsey, *Biophys. J.* **8**, 842 (1968).
14. Properly it measures curl J, but in simple axon geometries, Stokes's theorem is used at once (*3*).
15. R. K. Hobbie, *Intermediate Physics for Medicine and Biology* (Wiley, New York, 1978), pp. 178–203.
16. We thank J. K. Wikswo, J. Madey, E. Iufer, R. Debs, and J. Barnes for their encouragement and H. Sadisky for the ferrite cores. Supported in part by grants from the Research Corporation and the Vanderbilt University Research Council, and by NIH biomedical research support grant RR07089-13.

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Localization of Lysyl Oxidase in Hen Oviduct: Implications in Egg Shell Membrane Formation and Composition

Abstract. *Lysyl oxidase activity was found in the isthmus (the membrane-forming region) of the hen's oviduct in a copper-rich region proximal to the shell gland. Desmosine and isodesmosine, cross-linking compounds associated with mature elastin, were found in hydrolysates of the shell membrane, confirming the necessity for lysyl oxidase in its biosynthesis. Shell membranes from hens fed a copper-deficient diet or a diet supplemented with β-aminopropionitrile had a reduced content of desmosine and isodesmosine.*

Although early studies classified the protein in egg shell membranes as keratin (*1*), recent investigations established that the membrane is composed in part of proteins resembling those in connective tissue. Upon acid hydrolysis, the material yields hydroxylysines (*2*), hydroxyprolines (*3*), and desmosines (*4*), suggesting the presence of protein components bearing a structural resemblance to collagen and elastin (*5*). Moreover, shell formation is frequently defective in hens fed diets deficient in copper (*6*) or supplemented with the lathrogen β-aminopropionitrile (BAPN) (*7*); both treatments affect connective tissue protein metabolism by preventing the formation of cross-links. The molecular target is lysyl oxidase, which catalyzes a specific oxidative deamination of lysines that is obligatory to formation of the cross-links. The requirement of copper (*8*, *9*) and the inhibition of the enzyme by BAPN (*10*) are well established. A recent study (*6*) showed that lysine residues in shell membrane proteins are oxidized less in copper-deficient hens than in copper-supplemented hens. The data suggest that the shell membrane is formed through a process not unlike that which forms other connective tissue proteins. Thus we hypothesize that lysyl oxidase is required in the biogenesis of shell membrane protein. Evidence is presented here to support this hypothesis.

Oviduct tissue was obtained from sexually mature hens at the onset of the laying period. Segments (2.54 cm) from the entire length of the tube were examined for enzyme activity. The segments were weighed, minced, and homogenized in buffer (0.12M NaCl and 0.015M potassium phosphate, pH 7.6). The tissue was isolated by centrifugation, resuspended in a urea buffer (4M urea in 0.01M NaCl and 0.002M potassium phosphate, pH

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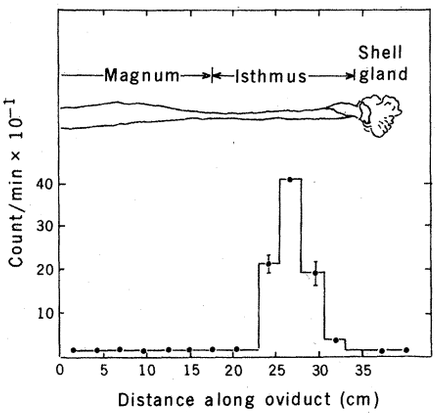

Fig. 1. Lysyl oxidase activity at different points along the hen oviduct. Enzyme activity is expressed as counts per minute of ^3H_2O released from L-[4,5-^{3}H]lysine-labeled aortic proteins (500,000 count/min) in a 4-hour assay at 40°C.

8.3), and briefly homogenized to extract the enzyme (*8*). All operations were performed at 4°C. After centrifugation, components solubilized by the urea treatment were dialyzed against phosphate buffer to remove urea. Lysyl oxidase activity in the extracts was measured with the procedures described in (*8*). Sections (1 cm) of isthmus tissue were bisected longitudinally so that half of each section could be tested for enzyme activity and the other half for copper content. The tissue samples were rinsed thoroughly with distilled, deionized water and then digested with a mixture of nitric and perchloric acids (wet ash procedure). Copper content was measured with an atomic absorption spectrometer.

The oviduct in a mature laying hen is a tubular structure ~ 65 cm in length, terminating proximally at the infundibulum and distally at the vagina. In between are the magnum, the isthmus, and the pouchlike shell gland. These are responsible for the formation of the albumen, the shell membranes, and the shell, respectively. Only the isthmus was found to contain lysyl oxidase activity (Fig. 1). Although the location of peak activity varied somewhat from oviduct to oviduct, it tended to be confined to the distal half of the isthmus and was sharply focused within a 3- to 5-cm section. The enzyme activity was strongly inhibited by a micromolar concentration of BAPN, confirming it to be lysyl oxidase.

That copper is present in oviduct tissue has been known for some time. Moreover, Moo-Young *et al.* (*11*) determined that the higher concentrations of copper are located in the isthmus section. We suggest that high concentrations of lysyl oxidase and copper share a common locale in the oviduct (Fig. 2). Since known lysyl oxidases in chick tissue are copper-dependent (*8*, *9*), the copper in the oviduct may be needed for proper enzyme action. It is not likely that the higher copper concentrations can be accounted for by the higher lysyl oxidase concentrations.

The finding of lysyl oxidase in the oviduct implies several possible biological functions for the enzyme. The enzyme could be necessary for the synthesis of the connective tissue proteins in the oviduct, which is itself composed mainly of smooth muscle cells and connective tissue. Lysyl oxidase is required for the synthesis of cross-links in the shell membrane protein, thereby giving stable but flexible organic support to the housing material before formation of the shell. Since the enzyme activity was confined to the membrane-forming region of the oviduct, the enzyme may have a role in a specific stage of shell membrane biogenesis (a more random distribution of the activity would have suggested otherwise). Furthermore, a close examination of the components in acid hydrolysates of membrane protein with three different chromatographic procedures (*12*) revealed the presence of desmosine and isodesmosine, the major cross-links in elastin (Table 1). The amount of each of these amino acids was reduced in shell membranes obtained from copper-deficient hens or BAPN-supplemented hens. Although elastin has not been isolated from egg shell membranes (*4*), our findings (i) support the involvement of an elastin-like connective tissue protein network in the synthesis of the shell membrane and (ii) support a mechanism in which the synthesis of the membrane protein involves an obligatory post-translational formation of cross-links in this protein. Cross-linking is initiated at a specific section of the isthmus before and during the passage of the egg to the shell gland. Failure to synthesize the cross-links affects the integrity of the shell membrane and leads to morphologically altered eggs (*6*).

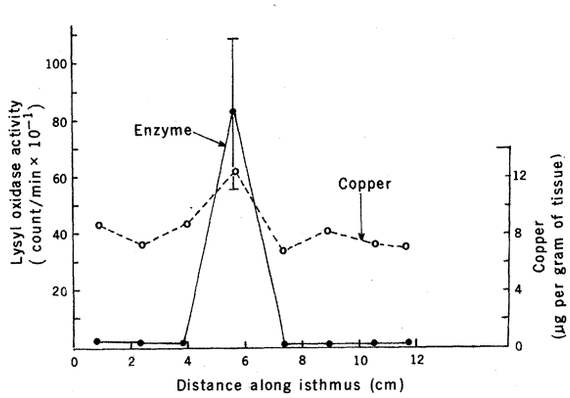

Fig. 2. Localization of copper and lysyl oxidase activity in the isthmus. Enzyme activity is expressed as in the legend to Fig. 1.

Table 1. Effect of diet on desmosine and isodesmosine content of egg shell membranes. Values are averages of two determinations.

Diet	Desmosine (μg/g)	Isodesmosine (μg/g)
Control*	367	189
Copper-deficient*	274	153
Commercial† plus 0.04 percent BAPN	319	182
	224	152

*Similar to diet used by Baumgartner *et al.* (*6*). †Commercial laying ration (Agway).

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References and Notes

1. H. O. Calvery, *J. Biol. Chem.* **100**, 183 (1933).
2. J. K. Candlish and R. K. Scougall, *Int. J. Protein Chem.* **1**, 299 (1969).
3. D. A. Balch and R. A. Cooke, *Ann. Biol. Anim. Biochem. Biophys.* **10**, 13 (1970).
4. R. M. Leach, Jr., and R. B. Rucker, *Poult. Sci.* **57**, 1151 (1978).
5. M. L. Tanzer, *Science* **180**, 561 (1973).
6. S. Baumgartner, D. J. Brown, E. Salevsky, Jr., R. M. Leach, Jr., *J. Nutr.* **108**, 804 (1978).
7. B. D. Barnett, D. J. Rickey, C. L. Morgan, *Proc. Soc. Exp. Biol. Med.* **95**, 101 (1957).
8. E. D. Harris, W. A. Gonnerman, J. E. Savage, B. L. O'Dell, *Biochim. Biophys. Acta* **341**, 332 (1974).
9. R. C. Siegel, S. R. Pinnel, G. R. Martin, *Biochemistry* **9**, 4486 (1970).
10. S. R. Pinnel and G. R. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 708 (1968).
11. A. J. Moo-Young, H. Schraer, R. Schraer, *Proc. Soc. Exp. Biol. Med.* **133**, 497 (1970).
12. B. C. Starcher, *Anal. Biochem.* **79**, 11 (1977); R. A. Green, J. A. Foster, L. B. Sandberg, *ibid.* **52**, 538 (1973); E. Moczar, B. Robert, L. Robert, *ibid.* **45**, 422 (1972).
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