The Copper Content of the Isthmus Mucosa and Certain Organs of the Domestic Fowl[1] (34505)

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The isthmus segment of the hen's oviduct which is involved in the formation of the shell membranes of the egg (1–3) contains an appreciable amount of copper (Cu) regardless of the location of an egg in the reproductive tract (4). This report is part of a study of the possible role of Cu in the synthetic function of the isthmus and deals with a comparison of the level of this metal in the isthmus mucosa with the levels found in other avian tissues. Since the glandular activity concerned with the formation of the shell membranes is located in the isthmus mucosa (5–7), it was of interest to ascertain whether the high Cu level found in the isthmus (4) is concentrated in the mucosa. Also, a paucity of information concerning the concentration of Cu in avian tissues and organs made it necessary to determine the Cu content of other organs taken from the same experimental animals for comparison with that of the isthmus mucosa.

Materials and Methods. Mature, laying, single-combed White Leghorn hens in their first year of laying were used throughout the study. Hens in various phases of the ovulation cycle were housed in wire cages and kept under artificial light for 14 hr/day. Water and a commercial ration were given *ad libitum.*

Each bird was decapitated and bled. The liver, kidney, lung, and isthmus were excised and trimmed of excess connective tissue. The liver, kidney, and lung were washed in cold 0.9% sodium chloride solution to remove excess blood. After two rinses with doubly glass-distilled water, which was used throughout these experiments, the isthmus was opened longitudinally, and the mucosa was separated from the serosa and muscle layers by scraping with glass knives. Washing and scraping procedures were performed in the cold at 0–4°. The tissues were placed in individual beakers, quickly frozen in liquid nitrogen (—196°) and lyophilized for 72 hr. They were then powdered in an agate mortar, placed in individual vials, stoppered, and stored over anhydrous calcium sulphate in a vacuum desiccator pending chemical analyses.

Nitrogen was determined according to the method of Ma and Zuazaga (8) and Cu by the method of Sunderman and Roszel (9) using a Perkin-Elmer Model 303 atomic absorption spectrometer. All glassware was acid-washed and rinsed at least three times with water. Care was taken to prevent contamination of the tissues with extraneous Cu.

Results. The data are summarized in Table I. The isthmus mucosa contained the highest amounts of Cu (33.39 ± 2.66 μg Cu/g dry tissue). A comparison of the mean Cu value of the isthmus mucosa with those of the other tissues shows that the isthmus mucosa contained approximately two, three, and five times as much Cu as the kidney, liver and lung, respectively. Statistical analysis shows that the mean Cu value of the isthmus mucosa is significantly higher ($p < .005$) than each of the other tissues studied.

To assess the difference between using dry weight and nitrogen content in expressing analytical values, the nitrogen content of the four tissues was also determined. If the Cu

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TABLE I. Copper and Nitrogen Contents of Certain Tissues of the Laying Hen.[a]

	Isthmus mucosa	Kidney	Liver	Lung
Micrograms copper per gram dry tissue[b]	33.39 ± 2.66 (11)	19.34 ± 0.60 (11)	9.91 ± 0.71 (11)	6.97 ± 0.53 (11)
Milligrams nitrogen per gram dry tissue[b]	133.66 ± 1.00 (11)	121.53 ± 2.41 (11)	61.15 ± 4.54 (11)	124.57 ± 1.39 (11)
Micrograms copper per gram nitrogen[b]	249.56 ± 19.94 (11)	160.38 ± 7.13 (11)	157.44 ± 7.69 (11)	56.11 ± 4.30 (11)

[a] Values presented as means ± standard errors. Number in parenthesis indicates number of experimental birds.
[b] In all cases, the difference between the mean values of the isthmus mucosa were significantly higher, $p < .005$, than those for the other tissues studied.

values are expressed on a nitrogen basis, the data show that the isthmus mucosa contains the highest amount of the metal (249.56 ± 19.94 μg Cu/g nitrogen) whereas the lung contains the lowest amount (56.11 ± 4.30 μg Cu/g nitrogen). There is no difference between the values reported for the kidney and liver, when expressed on a nitrogen basis. The differences between the Cu values of the isthmus mucosa and each of the other tissues were also significant at $p < .005$. The Cu level in the isthmus mucosa is approximately two times that of the kidney or liver and four times that of the lung.

The Cu value reported for the liver appears low in contrast to the other tissues studied and may be a consequence of the extremely fatty condition of the livers, which condition is reflected in the low nitrogen content of the organ.

Discussion. The heart of the domestic fowl contains 15 μg Cu/g dry tissue (10) while the liver contains 14.7 μg Cu/g dry tissue (11). Our results clearly indicate that the isthmus mucosa contains more Cu than the liver, heart, kidney, or lung. This suggests that the high Cu content is unique and necessary for the function of the isthmus.

Schraer and Schraer (4) reported an equivalent of 14.4 μg Cu/g dry tissue for the isthmus *in toto, i.e.,* including mucosa, serosa, and muscle layers. The present study shows that the isthmus mucosa contains approximately twice as much Cu as the whole tissue on a dry-weight basis. The serosa and muscle layers therefore contain little Cu. These results clearly demonstrate a concentration of the metal in the mucosa of the isthmus segment of the oviduct. Since most of the glandular activity concerned with the formation of the shell membranes is located in the mucosa (5–7) our results suggest that the high level of Cu may be closely associated with the specialized function of the isthmus, and may reflect a high concentration of cuproenzyme necessary for the formation of the shell membranes.

The shell membranes contain keratin-like material (12, 13). In the case of severe Cu deficiency in sheep, there is an increase in

the number of sulfhydryl residues and a concomitant decrease in the number of disulfide linkages in the wool keratin (1, 15). Further, it is believed that the oxidative closures of the sulfhydryl groups to give disulfide linkages is catalyzed by Cu (16). The possibility exists, therefore, that the Cu in the isthmus mucosa may be involved in the formation of the shell membranes by a mechanism analogous to the formation of elastin, which is mediated by a cuproenzyme, amine oxidase (17).

Summary. The distribution of Cu in certain avian tissues has been studied. The isthmus mucosa contains significantly ($p < .005$) more Cu than the kidney, liver, or lung, when expressed either on a dry weight or nitrogen basis. Since the isthmus mucosa is known to contain the glandular apparatus concerned with the formation of the shell membranes of the egg, it appears that the high Cu level in this tissue may be a reflection of a high concentration of some cuproenzymes(s) which may be necessary for the formation of the shell membranes.

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