

The Role of Chickens in Vitamin Discoveries

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Abstract: Animal models (bioassays) have been of great service to humanity in all sorts of scientific-related advancements. Aves, specifically chickens, played an important role in vitamin discoveries, as it was the first species to be used in 1890's to study beriberi. We briefly review its 30-year trajectory as an investigational tool in science and medicine.

Key words: Chickens, vitamin, bioassays

Introduction

Since time immemorial nutrition has been linked to health. Old preserved medical documents such as the ancient book of Thoth (ca. 3000 B.C.), the hoary Chinese herbal treatise (ca. 2600 B.C.), and the Egyptian Eber's papyrus (ca. 1552 B.C.) testify on ancestral knowledge of how foods can improve health, whereas the lack thereof leads to debilitating diseases. This became rapidly evident to seamen subjected to unbalanced, salt-loaded dried diets during lengthy trade and discovery voyages. Vasco da Gama, the valiant Portuguese explorer, is said to have lost more than 50% of his crew of 165 sailors to scurvy (then unrecognized as a vitamin C deficiency) in his 1498 voyage around Cape of Good Hope in Africa. Furthermore, throughout the Middle Ages, Siberia and northern Europe exhibited high prevalence of scurvy due to the lack of proper vitamin intake during treacherous winter months (Carpenter, 1986). In 1735, Spanish physician Gaspar Casal documented a medical description of a skin disorder he associated with consumption of spoiled corn and impoverishment. An epidemic disease characterized by dermatitis, dementia, skin lesions, diarrhea, weakness and insomnia similar to the one described earlier in France and Spain, swept through northern Italy; which prompted Milanese physician Francesco Frapoli to ascribed the term 'pelle agra' to the readily evident dermatological condition in 1771 (Roe, 1973; Sebrell, 1981). Pellagra, as it was later known, is a vitamin deficiency disease caused by dietary lack of niacin (vitamin B₃) and protein, especially proteins containing the essential amino acid tryptophan (McCollum and Simmonds, 1918; Goldsmith, 1958).

In the 17th and 18th centuries, rickets -a disturbance in mineral metabolism due partially to a vitamin D deficiency was highly prevalent in the State of New England, just as it had been in the European countries from where those settlers came from. In fact, official US records show that between 1910-1961 more than 13,000 deaths were attributed to rickets, 61% of these

occurring in infants under 1 year of age. It is believed that the first written medical account of the condition appeared in 1645 as D. Whistler's doctoral thesis at Oxford University. After learning about the low incidence of rickets among the people of the Baltic and North Sea coasts, it was concluded that cod liver oil prevented the disease, thus it became food remedy to treat adult rickets (Weick, 1967). Asian populations subsisting on polished rice as a major dietary component have been historically linked to beriberi a nervous system ailment caused by thiamin deficiency. During 1860's-1870's, Japanese doctors pinned beriberi to diet, and through ration reconfigurations to increase vegetables, barley and meats they were able to drastically reduce the incidence of beriberi, especially in sailors (Chick, 1975). Even though chickens have not been involved in all vitamin discoveries, they have played a preeminent role in the elucidation of water-soluble vitamins due to their rapid biological responses to nutrient deficiency and supplementation (Rosenfeld, 1997).

Chickens and the discovery of vitamins: Domestic chickens (*Gallus gallus domesticus*) have proved scientifically useful now and throughout history in serving as animal research models for studying diseases, nutritional hypothesis, immune responses and a vast array of applications that are now useful in human and animal health around the world (Combs, 1992).

Various food categories, as part of a nutritious diet, provide usable energy and heat to animals and humans through intestinal degradation and biochemical transformations, while all sorts of other compounds serve unlimited metabolic functions to ensure survival and proper physiological functions of organs. One of these categories is fruits and vegetables, which have been known to be an excellent source of vitamins and natural carbohydrates; these two, are by themselves, enzymatic cofactors and glycolysis substrates, respectively, among other things. *Vitamins*, which

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comprise one-third of the categorical nutrients, was a term initially coined by Polish biochemist Casimir Funk (1912) to describe a 'vital amine' in rice husks that prevented some ailments, particularly beriberi. However, even before proposing a hypothesis for essential dietary nutrients, Funk built upon research findings based on anecdotal, empirical and experimental evidence prior to the publication of his work. It is with this condition, beriberi, that chickens had their initial contribution in vitamin discovery.

The historical account of how chickens began to be used as animal models in vitamin research will be presented in the chronological order of events following along the years when a vitamin discovery was proposed:

Thiamin (1906): It took the keen curiosity of Christian Eijkman, a Dutch military physician sent in 1886 as part of a team from The Netherlands to Javanese hospitals in the Dutch East Indies to find the cause and solution to beriberi. Initially, Eijkman believed the condition had a bacterial etiology, and inoculated rats with blood of patients to no avail. In his laboratory he noticed that caged chickens exhibited edema, twitching, neuritis and paralysis. These signs in chickens were similar to the ones displayed by beriberi patients and persisted for some months before it suddenly disappeared without explanation. After reviewing the fed diets, he noticed a change from unpolished (brown) to polished (white) rice due to a failed shipment. Set to prove his hypothesis between rice husks and polyneuritis in chickens, Eijkman was able to show that adding rice polishing back to the diets of beriberi-stricken chickens restored their health, and also that the minerals they contained were not responsible for their alleviatory value. Between 1890-1894 Eijkman exploited this chicken bioassay, but his health flailed and had to return home; however, Dr. Adolphe Vorderman continued his work in 1895 as human trials among prisoners in the island of Java. By late 1896, Dr. Gerrit Grijns confirmed Eijkman's work with chickens and proposed the first statement conducive to the vitamin theory (Carpenter, 2000). Once a bioassay to study beriberi was proposed and validated, Funk (1911) capitalized on this information to report on the chemical nature of the compound that cures polyneuritis in birds.

Vitamin C (1907): Human subjects respond favorably to fruits and vegetables against scurvy. *G. gallus* synthesizes ascorbic acid. The guinea pig served as the animal model.

Vitamin A (1915): Dr. George Wald, an American working in Berlin in the early 1930's dissected animal retinas (including chickens) to obtain a light-sensitive purple compound then believed to be vitamin A.

However, it was 300 frog eyes that proved more useful than chickens as they resulted in the isolation of rhodopsin, whose stimulation with light yields two proteins, opsin and 'retinene' that in turn yields retinol. This finding was significant, as it was not expected for a vitamin to participate directly in the physiological process of vision (Wald, 1935).

Vitamin D (1919): Elmer V. McCollum initially working for the University of Wisconsin moved in 1917 to Johns Hopkins University on a hunch that the antirachitic factor in cod liver oil was different than the previously discovered vitamin A. He aerated and heated this fish oil and tested it on rat and chick bioassays. Heating destroyed vitamin A (antixerophthalmia) activity but retained the antirachitic properties. He called this heat-stable factor vitamin D (Combs, 1992; McCollum, 1964).

Vitamin E (1922): As the third fat-soluble vitamin to be discovered, little is known about the use of chick bioassays for its discovery besides the fact that it played a pivotal role in the elucidation of thiamin, vitamin A and D. Evans and Bishop (1922) at the University of California worked with a gestation resorption preventive bioassay in rats. Two years later, Sure (1924) proposed a new vitamin specific for reproduction.

Niacin (1926): Chickens do not exhibit pellagra-like symptoms as humans. This was confirmed in 1915 by Joseph Goldberger, a U.S. Public Health Service officer put in charge to study pellagra cases in the south of the country. Dogs were used as bioassays.

Vitamin B₁₂ (1926): Chickens did not have a direct role in cyanocobalamin discovery but only in its isolation (Nesheim and Kratzer, 2005). Hematologist G. H. Whipple accelerated blood regeneration in liver-fed dogs made anemic by exsanguinations. Murphy and Minot (1926) confirmed his results as effective therapy for severe anemia.

Biotin (1926): Boas (1927) hinted on the nutritive properties of egg whites. Later, Kögl and Tönnis (1936) isolated an egg growth factor that protected rats against skin lesions and hair loss when fed raw egg whites. du Vigneaud *et al.* (1942) worked out its chemistry.

Vitamin K (1929): Henrik Dam, a Danish biochemist, while investigating whether chickens needed a dietary source of sterols, found them able to synthesize cholesterol; however, some birds developed internal hemorrhaging due to a failure of their clotting mechanisms. He soon found this condition to be prevented by giving them a yet unknown compound present in both green leaves and liver, but not by recently discovered vitamins. 'Koagulation' is the Danish word

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Table 1: Nobel Laureates and their Research on Vitamins

Year Awarded	Scientific Contribution & Recipient	Compound(s)
Nobel Prize in Chemistry		
	Synthesis of Vitamins	
1937	Walter Norman Haworth	Vitamin C
1937	Paul Karrer	Vitamin E
1965	Robert Burns Woodward	Vitamin B ₁₂
	Structure of Vitamins	
1937	Paul Karrer	Vitamin A & B
1938	Richard Kuhn	Vitamin B ₂
1957	Lord (Alexander R.) Todd	Vitamin B ₁₂
1964	Dorothy Crowfoot Hodgkin	Vitamin B ₁₂
	Isolation of Vitamins	
1928	Adolf Otto Reinhold Windaus	Vitamin D
1938	Richard Kuhn	Vitamin B ₂ and B ₆
Nobel Prize in Physiology or Medicine		
	Discovery of Vitamins and Vitamin-related Compounds	
1929	Christian Eijkman	Vitamin B ₁
1929	Sir Frederick Gowland Hopkins	Growth Vitamins
1934	George Hoyt Whipple	Vitamin B ₁₂
1934	George Richards Minot	Vitamin B ₁₂
1934	William Parry Murphy	Vitamin B ₁₂
1943	Henrik Carl Peter Dam	Vitamin K
1953	Fritz Albert Lipmann	Coenzyme A
	Isolation of Vitamins	
1937	Albert Von Szent-Györgyi Nagrapolt	Vitamin C
1943	Edward Adelbert Doisy	Vitamin K

Table adapted from nobelprize.org; accessed 9.3.2006 at http://nobelprize.org/nobel_prizes/medicine/articles/carpenter/table.html

equivalent to the English word coagulation, hence the name vitamin K (Dam, 1935).

Pantothenic Acid (1931): Norris and Ringrose (1930) published work using a chick bioassay that prevented skin lesions and poor feather development with liver extracts.

Folate (1931): An antianemic factor found in liver extracts, yeast, alfalfa and wheat bran was proposed by various researchers. It prevented severe anemia in monkeys and chick bioassays (Combs, 1992). At the University of Arkansas Medical School hog kidney, chicken pancreas, and rat liver enzymes increased pteroylmonoglutamic acid activity in microbes involved in single carbon metabolism (Mims *et al.*, 1944; Totter *et al.*, 1944).

Riboflavin (1933): Norris *et al.* (1930), a poultry researcher at Cornell, described a specific type of leg weakness or paralysis in growing chickens that could be prevented with a milk concentrate. This was the first description of the classic deficiency disease termed 'curled toe paralysis' that later became known as a principal sign of riboflavin deficiency in growing chicks.

Vitamin B₆ (1934): A rat bioassay was used to elucidate pyridoxine. This term was coined by György (1934) to describe pellagra-like dermatitis in rats. Five years later, Harris and Folkers (1939) successfully achieved the

synthesis of vitamin B₆.

The role of chickens as animal models in vitamin research can be traced back to the late 1880's as Dr. Eijkman attempted to solve an unknown medical condition. From then on, it took the collaborative work of research scientists, in diverse disciplines, at various institutions around the world to come up with the 13 vitamins we now know (Table 1). This historical account provides insight on how avian species have contributed to nutritional sciences in more ways that we have imagined. Additionally, it attests to the powerful abilities of observation, the passionate curiosity of investigators, and the increasingly compounding effect of sharing scientific knowledge.

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