Inbred mice fed only bee pollen

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Summary
We previously reported that three different inbred strains of mice (CBA/Ki, C3H/f/Ki and C57BI/Ki) survived in a healthy condition when fed only a bee pollen granules diet and drinking water for 365 days. Similarly, Sprague-Dawley rats showed comparable skeletal and organ growth and development when fed a similar bee pollen diet during a twelve-week period as compared to control animals fed a standard laboratory diet. It was the purpose of this study to determine how long the survival time of CBA/Ki mice could be extended beyond 365 days when fed only bee pollen granules and water as compared to controls. Control mice survived a mean of 477 days (389-548) with 100% diagnosed with renal amyloidosis at autopsy which characterized this strain of mice in our laboratory. All pollen fed mice appeared healthy when euthanized at 600 days of age. Survival times were compared with a log rank test (p < 0.001). Also, there was no evidence of pathology particularly in the kidneys. These unexpected findings could be interpreted as being consistent with the genetotrophic disease concept proposed more than fifty years ago, namely, that bee pollen contains either a unique nutrient or a higher level of one or more nutrients that may be lacking or at a lower concentration in the standard diet which will then circumvent partial genetic blocks in the metabolic assembly line. If correct, this finding could provide an experimental model for study in the emerging field of nutrigenomics.

Keywords: bee pollen, diet, inbred mice, longevity, nutrigenomics.

Introduction
We previously reported that three different strains of inbred mice (CBA/Ki, C3H/f/Ki and C57BI/Ki) survived in a healthy condition when fed only a bee pollen granules diet and drinking water for 365 days (Liebelt et al., 1994). Similarly, weanling Sprague-Dawley rats showed comparable growth and development when fed a similar bee pollen diet during a twelve-week period as compared to control animals fed a standard laboratory diet (Liebelt & Calcagnetti 1998).

The products of the beehive, primarily bee pollen and honey, have been part of man's diet in various areas of the world for centuries (Devlin, 1993) and are essential for the survival of the beehive colony (Schmidt & Buchanan, 1992). Chemical analysis of bee pollen reveals the presence of a wide range of biochemically and nutritionally important substances including: minerals, trace elements, carbohydrates, organic acids, lipids, sterols, nucleic acids, proteins, free amino acids, water and lipid soluble vitamins, over 100 different enzymes and cofactors, Vitamin E, betacarotene and probably others (Vivino & Palmer, 1944; Stanley & Linsken, 1974; Schmidt & Buchanan, 1992; lannazzi, 1993). The purpose of this study was to determine the length of time male and female CBA/Ki mice could survive beyond 365 days of age when fed only bee pollen granules as compared to control mice fed a standard laboratory diet and then to compare any pathologic changes occurring in the two groups.

Materials and methods
Virgin CBA/Ki mice (ten males and ten females) were started on a diet of only bee pollen granules ad libitum and drinking water at 20 days of age. Prior to weaning at 20 days of age, these animals were fed a standard laboratory diet (LABLOX - Purina Lab Chow). A similar number of virgin CBA/Ki mice from the maintained breeding colony were continued on the standard laboratory diet. All animals were maintained in the Comparative Medicine Unit of the Northeastern...
Ohio Universities College of Medicine. The animals were housed in individual plastic cages at the same colony temperature (22°C) and lighting schedule (12 hours light and 12 hours dark). Standard glass feeding jars to minimize spillage were filled six days a week with either bee pollen granules or pulverized Lablox pellets so as the particles were of a similar size as the bee pollen granules. The mice were autopsied by the principal investigator at either the time of death or when euthanized according to standard autopsy procedures for our laboratory including tissue samples of the kidneys for microscopic study.

Result

The inbred strain CBA/Ki mice used in the present study had been bred and studied for over 20 years in our laboratory and has been characterized as a "low-cancer strain" in terms of developing spontaneous neoplasms (Liebelt, 1978). All control mice in the present study died between 389 and 548 days of age with one female diagnosed with a reticular cell neoplasm at 460 days of age and two males developed hepatomas at 495 days and 490 days of age. However, all control mice at autopsy revealed renal amyloidosis at the time of death, which has characterized this strain of mice in our laboratory (Fig. 1 A and B).

All the bee pollen fed mice survived in an apparently healthy condition to 600 days of age at which time they were arbitrarily euthanized for logistical reasons and autopsied. Survival times were compared with a log rank test (P < 0.0001). None of the bee pollen fed mice developed any type of neoplasm nor manifested any gross pathology or microscopic evidence of renal amyloidosis (Fig. 1 C and D).

Discussion

The earliest report found concerning the healthy survival of laboratory mice fed only bee pollen was a comment in a treatise by Caillas (McCormick, 1960). Preliminary reports by Burkholder and

![Fig. 1](image-url). Microscopic section of a CBA/Ki male mouse kidney at 500 days of age fed a standard diet (A) and a renal glomerulous from the same section revealing amyloid infiltration (B). Section of a CBA/Ki male mouse kidney at 600 days of age fed only a Bee Pollen Diet (C) showing no pathologic changes (D). The photo micrographs were stained with H & E.
colleagues (1987, 1991, 1993) concluded that inbred mice of a high mammary tumour strain could be maintained in good health and free of mammary tumours for extended periods of time when fed bee pollen as the sole source of nutrition. Our own studies demonstrated that a diet of only bee pollen granules provided the necessary nutrients for growth and development of mice and rats comparable to animals fed a standard laboratory diet (Liebelt et al., 1994; Liebelt & Calcagnetti, 1998). Also, the results of the present study demonstrated that bee pollen fed mice actually survived longer and remained free of any gross or microscopic evidence of renal amyloidosis in contrast to the control mice fed the standard laboratory diet.

These somewhat unexpected findings in the bee pollen fed mice brought to mind a concept proposed more than fifty years ago - "the genetotrophic disease concept" (Williams et al., 1950). A genetotrophic disease was defined as one in which the genetic pattern of the affected individual calls for the augmentation of a particular nutrient(s) and in its absence there develops a disease as a result of a nutritional deficiency. The concept focused upon the presence of partial genetic enzyme blocks somewhere along the metabolic assembly line that are probably commonplace in the inheritance of individuals (Beadle & Tatum, 1945; Mitchell & Houlahan, 1946, 1947). These blocks may give rise to increased requirements (under nourishment) for specific minerals, vitamins, amino acids or other nutritional factors, allowing for one individual to develop a nutritional disease when his/her diet is wholly adequate for many other individuals. A study on the appetites of rats and mice for alcohol (ethanol) led to the genetotrophic disease concept (Williams et al., 1949). Essentially, it was found that the patterns of drinking either alcohol containing water or plain water kept on a standard laboratory diet appeared to be under genetic control, but changes in the diet (supplemented or restricted) resulted in drinking patterns being shifted by nutritional means from one extreme to the other in consumption of the alcohol containing water. R.J. Williams went on to speculate that diseases such as cancer, rheumatoid arthritis, diabetes mellitus, mental illnesses and other chronic illnesses may also have a genetotrophic origin (Williams, 1956). Experimental studies designed to support this concept have not been found in the literature. However, a study described as an "exploratory clinical study" was carried out on a series of mentally retarded children receiving nutritional supplements and demonstrating improvements in IQ's (Harrell et al., 1981). More recently, it has been reported that multivitamin supplements delay the progression of HIV disease (Fawzi et al., 2004).

The concept also appears to be indirectly supported by the studies of Richter and colleagues (Richter & Eckert, 1937; 1938; 1939) using the nutritional "self-selection" method and concluding that animals "eat to live." In our own studies, we found that CBA/Ki mice, when presented with a choice between bee pollen granules and pulverized Lablox pellets after being weaned from the latter diet at 20 days of age, preferred an average of 72% (62-88%) of their daily caloric intake for the first two weeks from the bee pollen granules and then stabilized during the next four weeks at an average of 64% (58-76%) of the daily caloric intake from the bee pollen granules (Liebelt, 1997).

Regarding the development of renal amyloidosis in CBA/Ki mice, the earliest description of the pathological anatomy of the mouse kidney was that of Dunn (1944). During this same time period, several authors reported on the occurrence of spontaneous amyloidosis infiltrations of the kidney and other organs in several inbred strains of mice (Heston & Deringer, 1948; Kirschbaum, 1944; Kirschbaum & Bell, 1947) with a more recent and extensive study being carried out in "senile NYS mice that developed spontaneous renal amyloidosis." (Shimizu et al., 1993) The mechanism(s) involved in amyloid formation in all types of amyloidosis is apparently unknown but a recent report related to a possible mechanism for the deposition of amyloid in the brains of Alzheimer patients has been identified (Mambule et al., 2000; Lustbader et al., 2004).

The absence of renal amyloidosis in CBA/Ki mice at 600 days of age fed only bee pollen granules while all control mice dying between 389-540 days of age developed renal amyloidosis appears to be consistent with the genetotrophic disease concept. It would appear that bee pollen contains either a unique nutrient or a higher level of one or more nutrients that may be lacking or at a lower concentration in the standard laboratory diet, which prevents the development of spontaneous renal amyloidosis. The possibility that some factor was present in the standard laboratory diet that accounted for the amyloidosis appears to be a remote possibility at best.

More recently attention has been focused upon a paradigm shift in "understanding function in nutrition, health and disease" in the emerging field of nutrigenomics (Hyman, 2004). Nutrigenomics is explained as the interface between the nutritional environment and the cellular/genetic processes, namely, how common dietary chemicals can influence the health of an individual by influencing the individual's genetic makeup (Kapur & Rodriguez, 2004). Recently a detailed review article has proposed the future goals and strategies of the field of nutrigenomics (Muller & Kersten, 2003). The conceptual similarities between the "genetotrophic disease concept" proposed over 50 years ago and the more recently described "nutrigenomic concept" reflects rather vividly the profound shift from our understanding and interest in the physiology of nutrition at the whole animal level to the molecular level as regulated by genetics. The results of the present study if the interpretations of the findings are correct could provide an experimental model for the further study of nutrigenomics.

Whether the absence of spontaneous renal amyloidosis in bee pollen fed mice reflected a complete prevention of the disease or
rather, a delay in the time of appearance will require further studies. Additional studies utilizing other inbred strains of mice that are characterized by developing various strain specific diseases and fed only bee pollen diets are needed. Likewise, studies to determine the level of bee pollen supplementation to a standard laboratory diet are being pursued. Finally, whether this study has implications on defining the etiology of amyloid deposits in the brains of Alzheimer patients as being consistent with the genotrophic disease concept remains to be determined. These observations also raise questions of concern regarding the long-term consequences of the numerous restrictive "weight loss" diets now in vogue.

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