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IHD from copper deficiency: a unified theory

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Abstract

The theory, in brief outline here, implicating deficiency of Cu in the aetiology and pathophysiology of IHD explains more attributes of the disease than any other theory. This theory satisfies several of Hill's criteria of a half-century ago for deducing association between an environmental feature and presence of an illness. Most important is the temporal association between the rise of IHD and the decrease in dietary Cu since the 1930s along with a parallel increase in the supplementation of pregnant women with Fe, a Cu antagonist. There are more than eighty anatomical, chemical and physiological similarities between animals deficient in Cu and individuals with IHD. Few of these similarities have been produced by other dietary manipulations because feeding cholesterol induces Cu deficiency in animals. The most recent of these to be identified is decreased serum dehydroepiandrosterone. Some concomitant aspects of Cu metabolism and utilisation have been identified in other theories about heart disease: fetal programming, homocysteine, and Fe overload.

Key words: Alcoholic beverages: Atherosclerosis: Cholesterol: Paraoxonase: Superoxide dismutase: Coronary heart disease: Ischaemic heart disease

Introduction

Deaths from diseases of the heart did not exceed those from tuberculosis in the USA until 1910[1]; somewhat earlier the leading causes were pneumonia, tuberculosis and enteric disease[1,2]. The rise of heart disease in the 20th century has been chronicled; IHD was, by far, the leading cause of death in the USA by 1973[3,4]. The aetiology of this illness remains mysterious, although studies of migratory populations reveal it is environmental, not hereditary[5]. There are numerous causes of acute myocardial infarction but the leading cause, by far, is atherosclerosis of the coronary arteries[6]. Atherosclerosis has been found in anatomical samples from the tombs of the pharaohs[7,8].

In 1965 Sir Austin Bradford Hill[9] offered nine viewpoints regarding deducing causation from an associated environmental feature and presence of an illness. His fourth point was 'temporality', i.e. the temporal relationship of the association. Considering temporality along with his fifth point 'biological gradient' one can deduce that as exposure to the putative agent changes with time, risk of disease should change as well.

Here I examine the temporal changes in Cu in the Western diet as related to the epidemic of IHD along with a concomitant increase in supplementation with Fe, a Cu antagonist. These changes are complementary to, and consonant with, other viable theories on the origin of IHD. Some of the experimental evidence for the Cu deficiency theory also is summarised.

The lipid hypothesis

International comparisons of risk of IHD and some dietary characteristics have led many to believe that dietary fat is poison and that IHD is a slow and progressive intoxication. However, when similar epidemiology is done within single nations where environmental and social conditions are more homogeneous than between nations, the association between dietary fat and heart disease risk has not been found[5]. As heart disease risk was increasing in the 20th century, the apparently parallel increase in fat intake was found to be an artifact[10].

Nearly fifty epidemiological studies have found no association between dietary fat and heart disease risk or serum cholesterol[5,11]. For example, 'The failure to turn up any positive association between food intake and serum cholesterol level in the Framingham Diet Study led to the evaluation of a large number of variant analyses. These were uniformly unsuccessful in finding expected relationships'[12]. 'No dietary variables … had important or consistent associations with serum cholesterol …'[13]. Some have suggested that lack of evidence for this relationship should not dissuade us from believing in this relationship[14,15]. Diets of nearly 6500 men were modified to improve lipid metabolism, but after 6 years neither mortality from CHD nor total mortality was different from the control group[16].

Mann[17] reviewed the origins of the lipid hypothesis beginning in 1950, early dissention, some cholesterol-lowering trials involving diet or drugs and declared the 'end of an era' prematurely. He illustrated the lack of effect of cholesterol treatment on total mortality in anticipation of recent results. Even now decreasing cholesterol has a much bigger effect on the *way* that you die than on the *day* that you die because prolongation of life by statins is negligible[18].

Abbreviation: EAR, estimated average requirement.

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The copper deficiency theory

Development of the Cu deficiency theory on the aetiology and pathophysiology of IHD began when absolute or relative deficiency of Cu produced hypercholesterolaemia in rats[19,20]. Hypercholesterolaemia from Cu deficiency has been confirmed in at least thirty independent laboratories[21].

In summary

The Western diet often is low in Cu[22] with substantial numbers of individuals eating less than recommended amounts; Cu intakes in nutrition surveys are falsely high because calculated values greatly exceed values from chemical analyses. Cu deficiency is the only nutritional insult to experimental animals that elevates cholesterol, blood pressure, homocysteine, isoprostanes and uric acid, has adverse effects on arteries and electrocardiograms, decreases dehydroepiandrosterone, impairs glucose tolerance and paraoxonase activity, promotes thrombosis and oxidative damage, and to which males respond differently than females.

More than eighty anatomical, chemical and physiological similarities between animals deficient in Cu and people with IHD have been identified. Most of these similarities have been tabulated[23,24]. Data also were tabulated from more than sixty medical publications about more than 2500 people with poor Cu nutriture in cardiovascular, musculoskeletal and neural diseases with more than 1000 people benefitting from supplements containing Cu[22,25].

Anatomy, chemistry, pathology and physiology

More than ten of the more obvious clinical aspects of IHD are enumerated above. Examination of the anatomy, chemistry, pathology and physiology of atherosclerosis and IHD reveals a bewildering array of other, more subtle signs. Arteries show smooth muscle cell proliferation and hearts have ventricular aneurysms. There is decreased leucocyte Cu and increased cardiac Na along with heart blocks and hearing loss, *inter alia*. Some of these findings are exacerbated by salt or pregnancy; some are mitigated by aspirin or beer[23,24]. Cu deficiency has produced all of these findings in experimental animals. No other nutritional insult has produced such multifarious pathology in experimental animals.

Cholesterol probably is the dietary component tested most widely in the study of atherosclerosis and lipid metabolism. All of the thousands of experiments done since 1913[26,27] have Cu metabolism as a hidden variable because dietary cholesterol, or cholesterol plus cholic acid, induces Cu deficiency[28,29] according to several experiments from four, independent laboratories.

Copper nutriture

According to the *Oxford Textbook of Medicine*[30], low nutrient intake can reduce nutrient concentrations in tissues and compromise metabolic pathways. Tabulations[22] of medical articles are available on impaired Cu nutriture in cardiovascular, mostly ischaemic, diseases. Eleven articles on 254 individuals reveal low tissue Cu. Eight articles on 876 individuals reveal low activities of Cu-dependent enzymes indicating compromised metabolic pathways.

In addition, abnormal cardiac physiology is associated with poor Cu status. Oster *et al.*[31] reported that cardiac output correlates positively with cardiac Cu in patients with CHD. Erythrocyte superoxide dismutase was decreased in patients examined 6 months after myocardial infarction[32] and in patients with coronary slow flow[33]. Cu/Zn superoxide dismutase protects against oxidative damage and depends on Cu for activity[34].

Nutrition surveys

Nutrient intake data in large nutrition surveys are based on the memory of individuals interviewed about amounts and types of foods recently consumed. Their intakes are calculated from data obtained from food tables[22]. Eleven, peer-reviewed articles have been found in which the authors obtained the foods identified by survey subjects and measured Cu in them by chemical analysis. Intakes calculated from food tables exceeded these measured intakes in ten of the articles ($P=0{\cdot}0054$ by sign test)[35]. Thus there is a systematic, or determinate, error in the estimation of Cu intakes by calculation giving results that are falsely high by 77 % for Western diets[22]. Chiplonkar & Agte[36], Nakasuta *et al.*[37] and Rahmdel *et al.*[38] published the more recent reports since these concepts were considered in greater detail[22,39].

The RDA for the USA and Canada and estimated average requirement (EAR) for Cu are 0·9 and 0·7 mg daily, respectively[40]. Correction of published intakes (Third National Health and Nutrition Examination Survey (NHANES III)) for the 77 % error reveals that nearly one fourth of the women do not achieve the EAR and only half the men achieve the RDA[22]. Even fewer individuals achieve the higher recommendations (>1·0 mg/d) of the UK, European Commission, or Australia and New Zealand[22].

Decreasing dietary copper

Measurements of Cu in common foods published in 1942 were compared with measurements published in 1966; it was concluded that Cu had decreased in a quarter of a century[41]. The most robust data on this decline in food Cu[42] are from analyses made on archived wheat grain; a single analytical method revealed that Cu in the grain decreased since the mid 1960s.

In addition there was a decline in Cu in fruits and vegetables in the UK between 1940 and 1991[43], in fruits and vegetables in the UK between the 1930s and the 1980s[44] and in the USA since the 1930s[44]. Cu also declined in meat and some milk and cheese products in the UK[43] between 1940 and 1991. Fan *et al.*[42] also quote two surveys from the UK showing that Cu intakes fell in the last 15 years of the 20th century in agreement with tabulated percentile data from three surveys in the USA[22,40]. Even though some of these comparisons are separated widely in time, competent chemists probably produced accurate data earlier because analytical standards of excellence have not changed in a century. Thus IHD has increased while dietary Cu has decreased in agreement with Hill's viewpoint on temporality.

People deficient in copper

Approximately thirty-five men and women of middle age have been depleted of Cu in metabolic wards where experimental

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Table 1. Insufficient amounts of dietary copper for adult humans*

Amount (mg/d)	Criterion
0·65	Hypertensive response to handgrip test
0·78	Decreased glucose clearance
0·83	Hypercholesterolaemia
1·02	Lipoprotein change

* Modified from Klevay[108].

conditions were controlled carefully and dietary Cu was measured by analytical chemistry. Cardiac function was monitored with many Holter cardiograms. Data[45–48] are shown in Table 1.

Hypertensive responses to sustained handgrip exercise in women depleted of Cu fell below control values after repletion with Cu[45]. Cu depletion increased the concentration of plasma glucose by an average of 38 mg/dl (2·11 mmol/l) at each point on intravenous glucose tolerance tests done on two men; supplementation generally decreased these values[46].

Cholesterol in plasma of a young man was increased significantly by Cu depletion. He was supplemented with Cu when we noticed a short run of ventricular tachycardia; cholesterol then fell significantly and precipitously to less than the initial value[47].

In a similar experiment, Cu depletion increased LDL-cholesterol and decreased HDL-cholesterol. Four men were removed from the study because of one myocardial infarction, two severe tachycardias and an intermittent, second-degree heart block that were recorded[49]. No problems related to heart function were found in the previous 337 subjects observed during nutritional studies not involving Cu at that centre. Overall, lipoprotein cholesterols improved over control values on supplementation[48].

These lipid effects probably were driven by hydroxy-methylglutaryl-coenzyme A reductase (*EC* 1.1.1.34). Three independent laboratories have found its activity to be increased in rats deficient in Cu[50–52]. Activity of other enzymes germane to heart disease mechanisms generally is decreased[53]; some of these are mentioned here. No data have been found related to mechanisms for altered glucose metabolism or increased blood pressure. Some other interrelationships between Cu and heart disease are shown in Fig. 1.

More than 70 % of chemically analysed, daily diets of randomly selected people in Baltimore, MD, contained less than 1 mg Cu[54]. The people above responded to diets similarly low in Cu with potentially hazardous changes in risk factors that disappeared on Cu repletion. Production and elimination of abnormalities in depletion experiments are the *sine qua non* of measuring nutritional requirements.

Supplementation with copper

Cu supplementation[22] of people has abolished cardiac arrhythmia[55], protected erythrocytes from oxidation[56], increased activity of superoxide dismutase[57] and decreased plasma homocysteine (see below). Recently DiSilvestro *et al.*[58,59] found increased serum caeruloplasmin in young women and middle-aged men and women given Cu. In the latter study superoxide dismutase in erythrocytes also was increased. A micronutrient supplement containing Cu improved

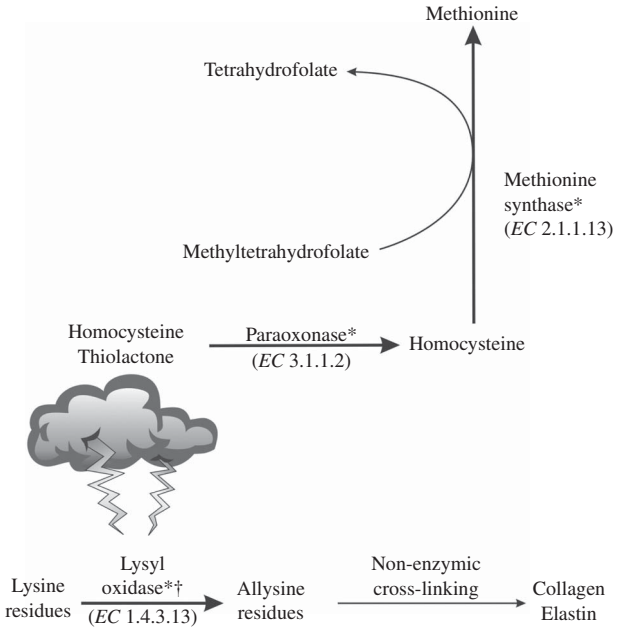

Fig. 1. Metabolic pathways affected by copper deficiency. For metabolic pathways involving homocysteine, also see the text. * Pathways inhibited by copper deficiency, in the figure and below. † Copper metalloenzyme, in the figure and below. The storm cloud and lightening bolts identify the inhibition of lysyl oxide† by homocysteine thiolactone. Superoxide dismutase*† (*EC* 1.15.1.1) defends against oxidative damage and is inhibited by high homocysteine. Homocysteine thiolactone and methyltetrahydrofolate are synthesised from homocysteine and *5,10-methylenetetrahydrofolate* by methionyl-tRNA synthase (*EC* 6.1.1.10) and *5,10-methylenetetrahydrofolate reductase* (*EC* 1.1.99.15), *respectively*. This figure was first published in Klevay[92] and is reproduced here with permission.

ventricular function in people with heart failure[60]. Cu supplementation (along with Zn) improved survival of people in a long-term, double-blind study of eye disorders[22,61]. According to Golden[30], a beneficial effect on a metabolic pathway or functional system from nutrient replacement is a sign of deficiency.

There are no large trials of Cu supplementation to reduce heart disease similar to those with vitamins to reduce homocysteine. It is possible that the failure of these trials to produce clinical benefit is because only vitamins that lower homocysteine were tested[62]. If Cu had been included in the supplemental mixture, it may have improved health by an unforeseen mechanism (see text below and Fig. 1).

Iron as a copper antagonist

Fe is known to interfere with Cu utilisation. Some early data were reviewed and an experiment revealed that higher dietary Fe can increase the dietary requirement for Cu[63]. High Fe intakes interfere with Cu absorption in infants[40] and adults[21]. Evidence that Fe overload interferes with Cu utilisation is increasing[64,65]. Haemochromatosis decreases immunoreactive caeruloplasmin[66]. Sometimes this interference is beneficial as when haemochromatosis delays the onset of Wilson's disease to the age of 50 years[67].

The onset of Fe supplementation of pregnant women was established by examination of *Williams Obstetrics* and

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advertisements in the *American Journal of Obstetrics and Gynecology* from the 1930s. John W. Williams was the founder of academic obstetrics in the USA[68]. His textbook first was published in 1903; several editions were examined.

The 7th edition recommends 'iron medication' as being 'very satisfactory' for the 'microcytic hypochromic anaemia of pregnancy'. Because the 6th edition does not mention Fe, one can infer that Fe supplementation during pregnancy began between 1930 and 1936. The 8th edition mentions ferrous sulfate specifically. The 24th edition[69] recommends '27 mg of elemental iron supplement be given daily to pregnant women'. Thus while dietary Cu was falling (above), use of a Cu antagonist in pregnancy was increasing, from no women in 1930, to some women in 1936, to all women now. Advertisement of Fe supplements began in 1933; a Cu–Fe supplement advertised for the anaemias of pregnancy in 1934 seems to have been abandoned.

Relationships with other theories

Kuhn argues that theories incorporating other theories contribute to scientific progress[70]. There are interrelationships between Cu deficiency and Fe overload, fetal programming and homocysteine.

Iron overload

Sullivan suggests that Fe overload contributes to heart disease risk[71–73]. It was suggested that people with Fe overload may benefit from Cu supplements because pathology related to CVD is resistant to phlebotomy[63]. Finland has abandoned Fe fortification of food, probably because of the associations of Fe with heart disease risk[63]. High intakes of haeme Fe were associated with increased risk of fatal, myocardial infarction in comparison with low intakes[74].

Pregnant women must consume an extra 0·75 mg of Cu daily to avoid depletion while supplying the newborn, placenta, etc., with Cu[75]. This amount plus the EAR (0·70 mg) is nearly 1·5 mg; approximately 60 % of 849 chemically analysed diets (from Belgium, Canada, UK and USA) contain less than this daily amount[76]. Supplementation with large amounts of Fe (a Cu antagonist, above), particularly if dietary Cu is too low, may harm both the mother and the baby later in life. Perhaps Fe supplements for pregnancy should contain Cu as in the mid-1930s (see above).

Fetal programming

Barker suggests that undernutrition *in utero* leads to IHD[77]. In brief, being small at birth and not catching up by the first birthday (or then being too fat) predisposes to diabetes mellitus or hypertension in middle age.

Barker suggests that 'deficiencies in specific nutrients which influence fetal growth, including vitamins A, C, and D, folate, Fe and Zn' may be important. Studies of nutrients as related to developmental models for adult disease seem to be few, but some data on Cu are available (see below). He cites O'Dell *et al.*[78]: rats made Cu deficient during gestation developed increased alveolar size, which persisted even with Cu repletion for several months[78].

Recent experiments on animals

Reproductive failure in animals from Cu deficiency has been known for more than a half century[79]. In female rats deficiency results in reproductive failure from fetal death on the 13th day of pregnancy[80]. Thus finding a low concentration of dietary Cu sufficiently high to provide offspring for study may be difficult. W. T. Johnson is prominent in this area; some anatomical, chemical and physiological abnormalities in pups born of deficient dams are resistant to extra dietary Cu across generations.

Female rats were fed a diet low in Cu for 3 weeks before mating and during gestation and lactation. Cardiac cytochrome c oxidase activity remained decreased in pups despite consumption of a diet adequate in Cu for 6 weeks[81]. According to Owen, the cardiac enzyme is particularly resistant to Cu therapy of deficiency[82].

A diet low in Cu was fed to female rats from 3 weeks before conception until 3 weeks after birth. Cytochrome c oxidase activity was decreased in cardiac mitochondria of adult offspring despite their being fed a diet with normal Cu from age 21 d to 290 d. Electron microscopy of cardiac myocytes was altered and H_2O_2 generation was increased in the replete animals[83].

A diet low in Cu was fed to female rats for 3 weeks before conception, during gestation and lactation[84] in a multigenerational study designed to evaluate effects of dietary remediation. Vascular functional responses in mesenteric arteries were examined using several agents; some sexual differences were noted. Impaired vascular function occurred in the second generation. Some effects were direct, from parents exposed to postnatal deficiency, and some were indirect from the initial *in utero* deficiency in spite of seemingly adequate Cu intakes in the first generation.

Although trans-generational effects of nutritional deficiency may not be unique to Cu, these experiments show that adverse effects of Cu deficiency *in utero* can lead to persistent pathology in offspring fed seemingly adequate amounts of Cu. Some adverse effects can pass to the next generation. The following observations on people are consonant with Cu deficiency *in utero* in people that Barker suggests will be at risk for heart disease in middle age[77].

Observations on people

Measurement of Cu in maternal plasma during pregnancy is assumed to be nutritionally irrelevant to fetal development because it increases markedly when the unborn are receiving substantial amounts of Cu from the mothers. Measurements on cord or infant blood, however, may be useful.

Krishnamachari & Rao[85] found that Cu and caeruloplasmin were halved in cord blood of infants born of malnourished mothers. Small, newborn infants had less serum Cu than normal[86]. Plasma Cu also was decreased in cord blood of infants small for gestational age at term and in premature infants whose size was appropriate for gestational age[87].

Superoxide dismutase activity was decreased in cord blood of small-for-gestational-age and preterm infants[88]. Premature infants have lower superoxide dismutase activity in erythrocytes

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and plasma after 100 d of life than term infants[89]; premature placentas are low in Cu and Cu-dependent enzymes[90].

These small infants, with impaired Cu status, are those that Barker suggests are likely to become hypertensive and/or diabetic in middle age. Diabetes mellitus and hypertension are major risk factors for IHD.

Homocysteine

McCully[91], cited by Klevay[92], suggests hyperhomocysteinaemia destroys arteries and promotes IHD. High homocysteine concentrations lead to increased homocysteine thiolactone, an irreversible inhibitor of lysyl oxidase, which depends on Cu to initiate the cross-linking of collagen and elastin in arteries[92]. Homocysteine thiolactone hydrolase, also known as paraoxonase, destroys this lactone. Its activity is decreased in rats deficient in Cu and low activity is associated with increased risk of IHD[1]. Cu supplements can decrease homocysteine concentrations in men[93].

Feeding homocysteine to rats decreases the activity of superoxide dismutase and disrupts Cu utilisation; rats deficient in Cu have increased plasma homocysteine and decreased activity of methionine synthase[92]. Methionine synthase may be a Cu enzyme. Some of these interrelationships are shown in Fig. 1.

Discussion

The epidemiology of IHD is filled with a bewildering array of apparently paradoxical observations. McCormick[94] considers multifactorial aetiology of heart disease to be a dangerous delusion. It has been suggested[1] that beriberi, pellagra and scurvy might have been considered multifactorial illnesses when their origins were as incomprehensible early in the 20th century as IHD has been in that century.

There is a temporal relationship between the rise in IHD and a fall in dietary Cu. A nearly simultaneous rise in Fe intakes during pregnancy also is found. Chamberlin[95], a contemporary of Hill, suggests that 'multiple working hypotheses' are useful in studying complex phenomena in promoting thoroughness and preventing neglect of important data. These concepts are related to the other theories on heart disease aetiology (see above).

Theories attempting to explain important natural phenomena have been found wanting historically if they explained only limited characteristics of the phenomena[96,97]. The Cu deficiency theory explains excess risk associated with gout and histidinaemia and the protective effects of cirrhosis and consumption of human milk in infancy[20,98]. Other theories do not include these observations.

There are numerous anatomical, chemical and physiological similarities between animals deficient in Cu and people with IHD (see above). The most recent chemical similarity between IHD and animals deficient in Cu to be identified is that related to dehydroepiandrosterone (DHEA)[99]. Low serum levels of DHEA predicted increased risk of CHD death among nearly 2500 Swedish men aged 61–89 years[99,100]; Cu deficiency halves DHEA in rats[101]. Other theories cannot explain many of these observations.

Barker revisited

Barker listed several nutrients (see above) that influence fetal growth as being of possible importance. Cu has only subtle effects on growth, such as on connective tissue in arteries. Few intergenerational experiments with nutrients other than Cu (see above) have been found. A mixed supplement given to improve offspring of rats malnourished during pregnancy and containing, *inter alia*, folate and vitamin C prevented hypertension, etc.; however, renal dysfunction and decreased glomerular number were unaffected[102]. Women with higher vitamin D concentrations in the first trimester were less likely to deliver infants that were small for gestational age[103]. Pre-term infants had less 5-methyltetrahydrofolate in erythrocytes obtained from cord blood than term infants[104].

Deficiencies of other nutrients may induce intergenerational effects. Although some nutrients have dietary associations, for example, Cu and folate[92], experiments with single-nutrient variables probably will provide the greatest clarity.

Sullivan revisited

Pregnant women who eat diets below the 60th percentile of those common in Baltimore, MD are not probably consuming sufficient Cu to supply a child and a placenta, etc. Consumption of extra Fe during pregnancy, which began to be recommended in the mid 1930s according to a major textbook, cannot have had a beneficial effect on Cu status. As Fe supplementation became more common later in the 20th century, it seems likely that adverse effects on Cu metabolism became more frequent. Cu deficiency from excess Fe is analogous (Hill viewpoint no. 9) to the well-known phenomenon of Cu deficiency from excess Zn. It is suggested that Cu supplements should always accompany Zn supplements[105]; this concept may be relevant to Fe supplements as well.

Hill revisited

Adverse temporal trends for dietary Cu and for Fe supplementation during pregnancy are found. Plausibility was Hill's sixth viewpoint. Similarities between IHD and animals deficient in Cu (see above) provide support for plausibility. Data summarised here also do not 'seriously conflict with the generally known facts of the natural history and biology of the disease' (coherence, viewpoint no. 7).

Enzymes related to the metabolism of collagen, elastin and homocysteine are mentioned above along with caeruloplasmin, Cu/Zn superoxide dismutase and cytochrome c oxidase. Statin drugs are designed to inhibit hydroxymethylglutaryl coenzyme A reductase; increased activity of this enzyme in Cu deficiency has been found in three independent laboratories[53]. Cu depletion has induced human hypercholesterolaemia[47].

Hill (viewpoint no. 8) admires experiment. A wide variety of cardiovascular abnormalities (which are among the numerous anatomical, etc., similarities, above) have been found in animals deficient in Cu. A supplement containing Cu improved heart failure[106]; a small, multicentre and similar trial may provide a test of one aspect of the Cu theory. More than ten medical articles reveal low Cu nutriture in CVD[22].

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The theory is a fruitful source of testable hypotheses about IHD that may involve Cu metabolism. For example, it is well known that moderate consumption of alcoholic beverages is protective against heart disease death. When rats are fed a diet deficient in Cu, those that drink beer live six times as long as those that drink water and have less heart damage and lower cholesterol[107]. Ethyl alcohol in a similar concentration as in beer had no benefit. Other experiments based on IHD have identified new aspects of Cu metabolism.

Conclusions

The Cu deficiency theory was examined in relation to Hill's classic viewpoints regarding deducing causation from an associated environmental feature and presence of an illness. There is a temporal association between the rise in heart disease and the fall in dietary Cu and with a rise in the supplementation of pregnant women with Fe, a Cu antagonist. The theory also satisfied Hill's other criteria of analogy, biological gradient, coherence and plausibility. A great many animal experiments, depletion/repletion experiments with men and women and some Cu supplementation trials in people are consonant with Hill's belief in experiment and with the Cu deficiency theory. There are common features between this theory and other theories about IHD involving fetal programming, homocysteine and Fe overload.

The Cu deficiency theory is the simplest and most general explanation of the aetiology and pathophysiology of IHD. No other theory explains such a variety of lesions or epidemiological observations. Cu deficiency is a hidden variable in the numerous experiments on cholesterol feeding that were meant to elucidate the atherosclerotic process leading to IHD. Fe supplements in general, and especially in pregnancy, should include Cu as in the 1930s.

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Some secondary references, which include many original sources, are cited in the bibliography to save space. For example, in the paragraph on cholesterol feeding near the end of the section on Anatomy, chemistry, pathology and physiology, one reference substituted for six others.

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