CYSTIC FIBROSIS; HETEROZYGOTE SURVIVAL IN A TOXIC ENVIRONMENT?

A Hypothesis to account for the high incidence of Cystic Fibrosis among persons of western europ̀ean ancestry

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ABSTRACT: Cystic Fibrosis (C.F.)is an unexpectedly frequent disease among persons whose ancestors originated in western Europe. Possibly because of its many symptoms, the cause of this autosomal recessive, genetic disease is unknown.

Measured abnormalities in calcium and sodium metabolism in the heterozygote population and in sodium, calcium and five other metals in the homozygote are indicators that other less readily measurable metals, including lead, may also be metabolized abnormally by homozygotes and heterozygotes.

The hypothesis is proposed that heterozygotes of C.F. enjoy an adaptive advantage which protects them against the chronic lead poisoning to which they and their ancestors were exposed during the last five millenia. This protection outweighs the severe fitness handicap imposed on homozygotes and has led to the unexpectedly high incidence of this disease in persons of european ancestry.

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History and Genetics of Cystic Fibrosis

"Oh there's one thing I can tell all right. I always know if babies are going to live or die. But that's easy. Right when they're born, as soon as the midwife washes them clean, I give them a lick from head to toe. And if they taste real salty, that means they're going to die. I've never been wrong. Not even once. Mothers are always sending for me, just so I can tell them...". (1)

"Das Kind stirbt bald wieder dessen Stirne beim Kussen salsig schmect." (2) The child whose brow tastes salty will die soon.

These quotations both precede the identification of cystic fibrosis by about 100 years, but the poor prognosis of children whose sweat is salty corresponds to the modern use of sweat sodium level to diagnose cystic fibrosis.

Fanconi in 1935 proposed that the symptoms -- disposition to respiratory infection, abnormally viscous secretions, malabsorption of food (especially fats), and elevated salt in the sweat which seemed to cluster in certain families was a single inborn error of metabolism which he called mucoviscidosis.(3)

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Within about 10 years of this suggestion, enough evidence had accumulated to show that cystic fibrosis (C.F.) as it is now called, is an autosomal recessive genetic disease which affects about one in 2000 newborn children of western european ancestry. Although a large volume of information is now available, treatment is still limited to relief of symptoms, the root cause is not known and carriers cannot be identified until their first affected child is diagnosed.

Such a high incidence as well as racial specificity are unusual in a genetic disease, however C.F. has been shown repeatedly to have an incidence of 1 in 1500 to 2500 live births in persons of western european ancestry, but only 1 in 17000 in American Negroes and only 1 in 90000 Mongolians. (4) Table 1 is a summary of the racial distribution of the incidence of C.F. taken from Wood, Boat and Doershuk. (5) The incidence among Finns (6) and Jews (7) also seems to be lower than in Western Europeans. The elevated carrier frequencies calculated from disease incidence among western Europeans suggests that the C.F. gene provides an adaptive heterozygote advantage to western european carriers in the same way that Sickle Cell Anemia has been shown to provide an advantage against Malaria to the carriers of that trait. (8)

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Table 1 Taken from Wood, R.E., Boat, T.F. and Doershuk, C.F. (5)

ESTIMATED MINIMAL INCIDENCE OF CYSTIC FIBROSIS (LIVE BIRTHS)

Incidence	Year of Report	Location	Reference
1:90,000	1968	United States (Hawaii, Orientals)	17
1:17,000	1974	Washington, D.C. (blacks)	18
1:15,000	1970	Italy	19
1:10,000	1974	England (Pakistani immigrants)	20
1: 8,000	1962	Sweden	21
1:4,000–8,000,	1971	European Soviet Union	22
1: 3,800	1968	United States (Hawaii, whites)	17
1: 3,700	1960	United States (Ohio)	23
1: 3,400	1966	United States (Middle and South Atlantic states)	24
1: 3,300	1963	Germany	25
1: 3,300	1972	Czechoslovakia	26
1: 3,200	1961	France	27
1: 3,000	1966	England	28
1: 2,900	1967	England	29
1: 2,600	1967	Czechoslovakia	30
1: 2,450	1966	United States (Buffalo, N.Y.)	31
1: 2,450	1965	Australia	32
1: 2,400	1968	England	33
1: 2,400	1962	United States (New England states)	34
1: 1,900	1962	United States (Indiana)	35
1: 1,860	1965	United States (Connecticut)	36
1: 1,000	1952	United States (Minnesota)	37
1: 620	1975	South West Africa (Dutch descent)	38

Cystic fibrosis; heterozygote survival

If the gene is at equilibrium, its frequency is many orders of magnitude beyond what could be maintained in the population by the occurence of fresh mutations. It is likely, therefore, that a high equilibrium frequency has been achieved by a selective disadvantage to the homozygotes balanced against a selective, adaptive advantage in favour of the heterozygotes.

If C.F. reached such a high incidence in western Europeans in response to an environmental hazard, what is the aspect of the disease, what is the environmental factor peculiar to western Europe and what is the relationship between them?

Metal-Ion Abnormalities in the Heterozygote Population

Only a few of the many characteristic abnormalities of C.F. are expressed in the heterozygote population, but those which are can be associated with abnormalities in sodium and calcium ions. Increased calcium binding of serum from C.F. heterozygotes has also been reported. (9) Abnormally elevated levels of the Mangos (10) and Spock (11) factors have also been reported in the serum, sweat or saliva of C.F. obligate heterozygotes. The Mangos factor is clearly related to abnormal cat-ion behaviour since retrograde perfusion of the Mangos or sodium resorption inhibitory, factor into normal sweat glands increases the sodium content of the resulting sweat. The Spock, or ciliostatic, factor disorganizes and retards the beat of cilia in rabbit tracheal explants and other ciliated tissue. This factor is also suspect as a source of metal ion abnormality, since calcium ionophore A23187 mimics its ciliostatic activity.(12,13,14)

The relationship between the abnormalities in sodium transport factor and calcium binding observed in C.F. heterogygotes and their adaptive advantage will be considered after a review of the more extensive abnormalities in metal ion metabolism observed in C.F. homozygotes.

Metal-Ion Abnormalities Among Homozygotes

The evidence, summarized in Table 2, shows that among homozygotes, sodium is only one of six metal ions present in abnormal amounts in the fluids and tissues.(15-38) The other metals are normally present present at such low levels in normal controls that it is difficult to state with conviction that they are present at a reduced level in C.F. patients. Nevertheless, manganese has been reported at a reduced level in hair, sweat and fingernails of C.F. patients. (15) Low levels of serum iron and zinc may be secondary to the recurrent respiratory infections to which C.F. patients are subject.(39) Also, during hot weather, excessive sweating and high sodium content of sweat occasionally deplete the serum sodium of C.F. patients .(40)

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TABLE 2. METAL-ION CONTENT IN C.F. FLUIDS AND TISSUES

	Na	K	Ca	Mg	Fe	Cu	Zn
Sweat	↑,↑	↑,↑	N,↑	↑,N	-	N	-
Saliva	↑,↑	↑,N	↑,↑	N	-	N,N	↑,N
Serum	N,↓	N,↓	↓,N	N	↓	N,N	↓
Tears	↓,N	N,N	↑,N	-	-	-	-
Urine	N,N,↓	N,↓	N,↓	N	-	N,N	↑
Duodenal Fluid	↑	↑	↑	↑	N	-	↓
Others	↑,↓	↑,↑	↑,N	-	-	-	-
Meconium	↓	↓	↓	↓	↓	↓	↓
Hair	↑,↑	↑,↑	FREE ↑ BND. ↓	↑,FREE ↑ BND. ↑	-	-	↓
Nails	↑,↑ ↑,↑	↑,↑	↑	↑,↑	-	↑	↓
Pancreas	↓	↓	↑	↓	-	↓	↓

↑-Elevated, ↓- Depressed, N-Normal, BND-Bound

Several symbols in the same position indicate the results of different authors.

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The difficulty of determining normal levels of trace metals is partly due to the difficulty in assaying parts per billion of these metals, but also due to the large potential for variations in the diet and other exposure from subject to subject. If the trend for the metals which have have been reported (table 2) extends to other metals as yet unreported then C.F. could be characterized as a generalized defect in metal ion metabolism. Copper is handled normally thanks to the specific control mechanisms which malfunction in Menkes syndrome and in Wilson's disease and which function normally in C.F. to override the generalized defect and maintain normal levels of copper.

Cystic fibrosis; heterozygote survival

Adaptive Advantage to Heterozygotes.

Suppose that heterozygotes excrete elevated concentrations of other metals in addition to sodium, and calcium. What possible environmental hazards specific to western Europeans would greater metal ion excretion mitigate? What survival advantage to the heterozygote would be offered by this increased capacity to excrete metal ions?

In ancient times only seven metals were mined and smelted: gold, silver, copper, iron, tin lead and mercury. Of these, gold, silver and mercury were and are rare. Copper and iron are accommodated by the body. Tin, while it is not normally handled by the body, is not especially toxic. Lead, by contrast, is not only extremely toxic, chronically and acutely, but also causes sterility and stands out among the metals which could cast metal excreters in the role of survivors.

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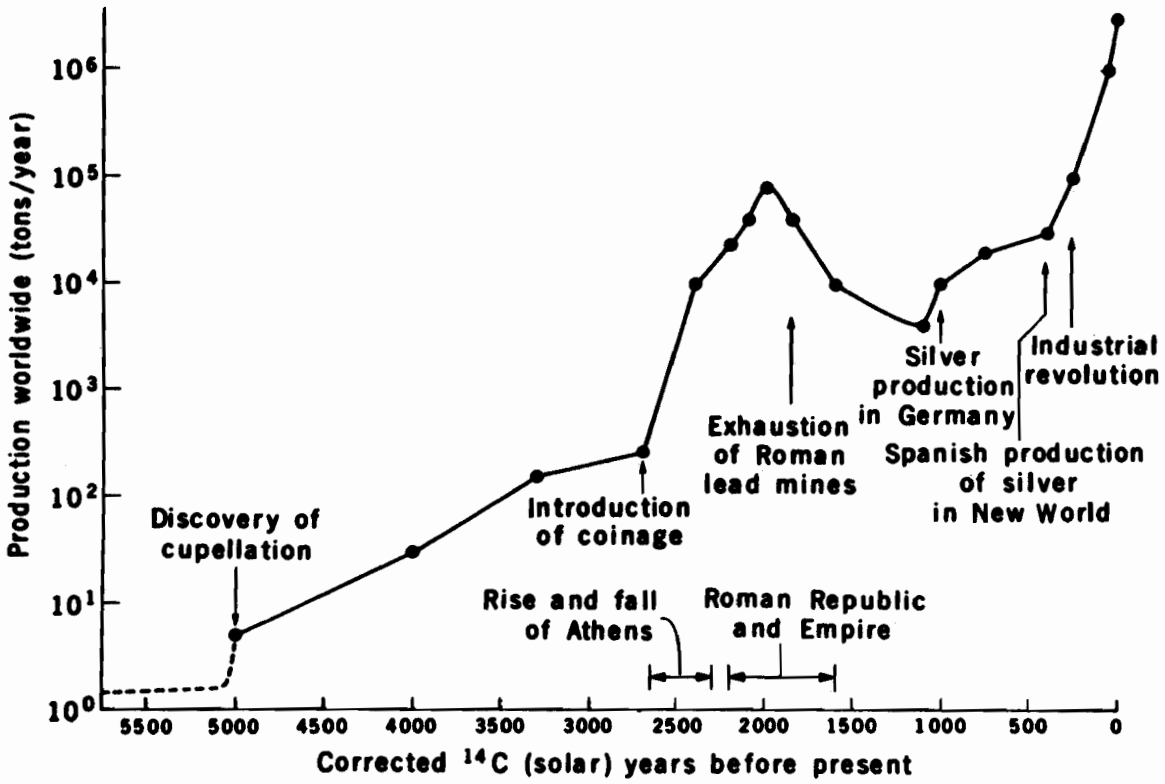

Figure 1 World lead production over the past 5500 years. Most of this production was in or exported to western Europe, the region where C.F. reaches its highest incidence.(41)

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The ways in which western Europeans were exposed to the toxic effects of lead varied during the five millenia depending on how it was used.

Initially, during the Roman empire the most serious exposure to plumbism was from lead plumbing. To avoid building expensive bridges for their aqueducts, the Romans exploited the principle of the siphon to let water run down one mountain and up another. The siphons were either made of lead or caulked with lead. Lead was also used to line copper wine vessels to prevent a bitter taste from developing in the wine. In Roman times lead salts were used as an abortafacient. Lead was also used in the glaze on ceramic dishes as well as in leaden cooking pots.

In two recent articles, Eisinger (42,43) has outlined the hazard of lead poisoning from antiquity and has documented the frighteningly widespread practice in medieval times of using lead acetate, or sugar of lead, as an artificial sweetener and preservative for food and drink. This practice which may have extended to rum and cider as well as wine is documented in France, Holland, Sweden, England and Germany -- just those regions where C.F. reaches its unexpectedly high incidence. If the lead was not introduced intentionally the lead components of the cider press or the condenser on the still provided a source of lead contamination. During the fifteenth century, the hazards of lead to potters, miners, painters and tipplers were recognized and measures were taken to reduce them, but new sources of exposure to lead developed as the older ones were controlled. Even today lead poses a hazard in the form of paint and putty in which children rediscover the sweetish taste of some lead compounds, in lead soldered tea kettles and food tins, industrial effluents in the air and water and automobile exhaust. (44) The severe lead intoxication which formerly reduced fitness by debilitation, sterility and death may have been reduced, but lead ingestion is still demonstrably implicated in hyperactivity and mental retardation, (45) and may now reduce fitness through these conditions.

In this connection it is interesting to note that tests on C.F. patients and their siblings indicate higher than normal I.Q. levels. (46)

It is possible that normal I.Q. levels are depressed by the total body burden of lead (500 fold as great as it was in the pre-lead era (44)) we now accept as normal. and the apparently high I.Q.s registered by the C.F. patients and their siblings simply reflect the norm for children whose lead levels are closer to historic lead levels?

It was mentioned earlier that C.F. heterozygotes appear to handle calcium abnormally and have elevated levels of a serum factor which resembles a calcium ionophore.

Cystic fibrosis; heterozygote survival

There is evidence that lead is apparently processed inadvertently along with calcium by virtually all organisms. (44) It is therefore not unreasonable to suggest the abnormality in calcium metabolism observed in the C.F. heterozygote population extends to lead and in some way offers protection against chronic lead intoxication.

It is possible to chart the increase in incidence of cystic fibrosis over the past five millenia by means of certain assumptions and equations 1 and 2.

It is assumed;-

1. That generation time is 25 years,
2. that the equilibrium incidence in antiquity was equivalent to the mutation rate squared = 10^{-5} (47, p246)
3. that the selective disadvantage of the wild type homozygote was constant during the 5000 years of exposure to lead, and
4. that the selective disadvantage of the mutant (C.F.) homozygote is 1.0.

Equation 1

$$Q_{n+1} = [Q_n + Q_n^2 \cdot (1 - S_2)] / (1 - P^2 \cdot S_1 - Q_n^2 \cdot S_2)$$

Equation 2

$$I = Q^2$$

where
P = proportion of wild type gene
Q = proportion of mutant C.F. gene,
= estimated mutation rate = 10^{-5} from disease incidence = 0.0004
S1 = the selective disadvantage of wild type homozygote relative to the heterozygote.
S2 = the selective disadvantage of the mutant homozygote (C.F.) assumed = 1. in the absence of treatment.
n = number of generation
I = incidence of C.F. in Europeans

It follows that the constant disadvantage to the wild type homozygotes is 4.4% relative to the heterozygote when exposed to an environment containing unnaturally high levels of lead and also that the incidence of C.F. has followed the course shown in Figure 2.

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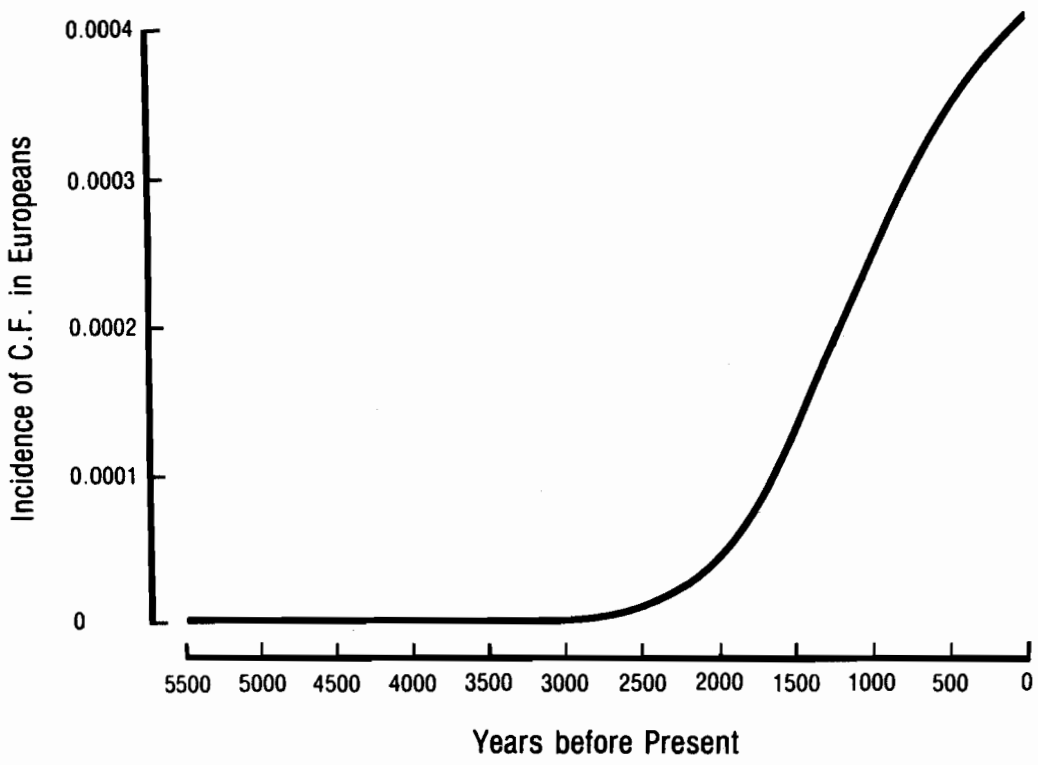

Figure 2. Incidence of Cystic Fibrosis over the past five millenia calculated from assumptions and equations in text.

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Only a slight increase in daily lead excretion or decrease in daily lead absorption would be enough to provide this protection. The lower gene frequencies for other populations may reflect shorter exposure to chronic lead intoxication.

By comparison , C.F. heterozygotes would require a selective advantage of 3% (48 p399) to maintain their high gene frequency in a state of equilibrium. Other reports which suggest an increase in fertility of the grandparents of C.F. patients, have proposed a selective advantage to the heterozygote of 10% (49) and 21% (50)

Formulation of hypothesis and suggestions for testing it.

I propose that C.F. reached its elevated incidence in Western Europe because the abnormality in metal ion metabolism observed in 7 metals in the homozygote extends to lead and in some way protects the heterozygote from the toxic effects of exposure to lead.

The obvious test of this hypothesis is to perform lead loading or clearance experiments on carriers and normal controls. Prospective experiments of this kind probably could not be carried out in an ethically acceptable way. However, it is possible that an inadvertant natural experiment might provide data which could be analysed retrospectively to provide a test for the hypothesis. For example, in a region where lead in the drinking water or in the environment generally was known to be present at an especially high level, the extracted first teeth of C.F. patients, siblings and normal controls could be assayed for lead by dissolution in 70 per cent $HClO_4$ and anodic stripping voltammetry. (51) The lead level of these teeth represents the cumulative history of the exposure to lead of the child. If the hypothesis is correct , the teeth of C.F. patients and their siblings would be expected to contain less lead on average than normal controls from the same region of high exposure to lead. A comparison of the I.Q.s of children with C.F., their siblings and matched controls from a region of high exposure to lead would be interesting, but inconclusive.

Batuman et. al.(52) are of the opinion that the E.D.T.A. lead mobilization test appears to be the most sensitive index of excessive lead absorption. This test is more invasive than an assay of extracted teeth, since it depends on administration of 1 gram of E.D.T.A. daily for at least one day, but it has the advantage that it could be used to compare adult obligate heterozygotes with matched controls. Results from regions of high exposure to lead would be the most meaningful.

Alternatively, zinc loading or clearance are ethically acceptable procedures.(53) If a population of heterozygotes showed a significant difference in their zinc clearance rate the hypothesis would be supported, but not demonstrated correct.

Conclusion

Eisinger (43) writes ".... (thanks to).... the biological defences with which evolution has endowed us. The level of copper, for instance, a toxic but abundant metal, is kept within narrow limits in everyone except sufferers of a rare genetic condition (Wilson's disease). Since lead, on on the other hand, occurred in very low levels until it was mined and smelted, a corresponding homeostatic system to control this toxin did not develop."

In this report it is proposed that a biological system to control the effects of chronic exposure to lead has already evolved, but that its devastating effects on the homozygotes have overshadowed the subtle benefits it still affords heterozygotes.

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