GENES AND ENZYMES

From the material we have already covered in this course you should have a good feeling for what a gene is, and how genes and simple traits can be passed from one generation to the next. However, even with a simple single gene trait, there are many more questions we can ask, some of which we still may not be able to answer. How do different alleles cause different phenotypes? What happens when a mutation occurs? What determines if and when a particular gene functions? Lets take a true example of a single gene trait to make this point. Polled (hornless) in cattle is dominant, and horned is recessive. How can the recessive pp genotype cause horns to grow, and why do they always come out at the right place? All the cells of a horned animal should have the same genotype, but they don't all have horns. What does the p allele do, and when and where is it expressed? In this case, I'm afraid I still can't tell you all the answers!

The next question we will approach, is "How does a gene work?" Naturally this means we will have to get down to the molecular level, and I know that this scares some of you. I will not deny that those who already know organic chemistry and/or biochemistry may have an advantage. Of course that is true in every course you take; the better the background, the easier the material will seem. But I will also assure you that we will cover everything you need to know in class, and that we will not be asking you to memorize a bunch of molecules or reactions. Over the last couple of semesters, the highest grades on these tests have come from an English major, a BUAD major and an AgDEV major. I doubt any of them had a super background, but I do imagine that they may have worked a little harder.

Our Goal for today is to establish a connection between genes and enzymes. We will take a historical approach. In one way, since humans are so complex and difficult to study, it is surprising that solid information first came from humans. On the other hand, since there was and is so much natural interest in humans and hereditary defects, perhaps we should not be at all surprised.

The work we will consider goes back to 1909, when Sir Archibald Garrod, an English physician, published a book entitled "Inborn Errors of Metabolism"

There used to be at least a couple of copies in our library, but I haven't checked lately; they may have been ripped-off as are so many things!

In his book, Garrod described a number of genetic anomalies that could be related to specific chemical reactions. As you can see from the publication date, he caught on to Mendel's concepts very soon after they were rediscovered, but his concepts in this area, like those of Mendel, were not really appreciated until sometime after his death. As we look back today, it is clear that he had a real grasp of the mechanisms of gene action and function.

One of the diseases Garrod studied was ALCAPTONURIA (= ALKAPTONURIA)

Alcaptonuria is inherited as a single gene defect; normals have at least one dominant allele and those affected are homozygous recessive.

LEGEND: A_ = normal
aa = affected (alcaptonuric)

Alcaptonuria is a rare disorder, but it is very easy to detect; it causes the urine to turn black when exposed to air. It's not so quick as to be embarrassing or anything, but it is something that mother's may notice about the diapers of their affected baby. This is how the cases are usually discovered. There are some other symptoms also; as the affected person ages there tend to be yellow deposits in the eye, and often there is a darkening and hardening of cartilage tissues, leading to an arthritis-like condition. Even so, it is not considered a very serious or threatening disease.

When urine from alcaptonurics was analyzed, it was found to contain a chemical compound that was not present in normals. This compound, homogentisic acid, (HA) is the substance that turns black when it is oxidized.

Legend: A_ = normal
aa = affected (alcaptonuric) (accumulate and excrete HA)

Garrod proposed that the reason that normals do not accumulate homogentisic acid is that they convert it to another compound called maleylacetoacetic acid, and furthermore, he suggested that the reason they could do so was that they have an enzyme that is missing in alcaptonuria. I'm sure that all of you have some idea of what an enzyme is, either from previous courses or even from commercials that have been on TV. However, I want to go on and write down a formal definition so we will all be on the same footing:
For our purposes:

An enzyme is a protein that catalyzes a specific chemical reaction.

A protein, as we will see later, is a chain of amino acids, and a catalyst is a substance that causes a reaction to proceed without itself being used up or converted in the reaction.

The names of enzymes usually end in **ase** ie, RNase breaks down RNA, proteases are useful in digestion because they breakdown proteins etc. The name of the enzyme that is present in most people but lacking in alcaptonurics is homogentisic acid oxidase.

Thus we can rewrite the legend:

A_ = normal -- have HA oxidase
aa = affected (alcaptonuric) -- lack HA oxidase

In this case we see that the dominant allele corresponds to the presence of one specific enzyme and that homozygous recessive individuals lack the enzyme, directly leading to the associated phenotype. Lets explore this a little further with some other genetic diseases that turn out to be related.

When alcaptonurics are fed high protein diets, they excrete more HA. In particular, the levels of two of the amino acids that are present in protein, phenylalanine and tyrosine, seem to correlate to HA production.

This brings us to a much more serious disease called **phenylketonuria or PKU**

PKU is also inherited as a **single gene recessive** character. ie

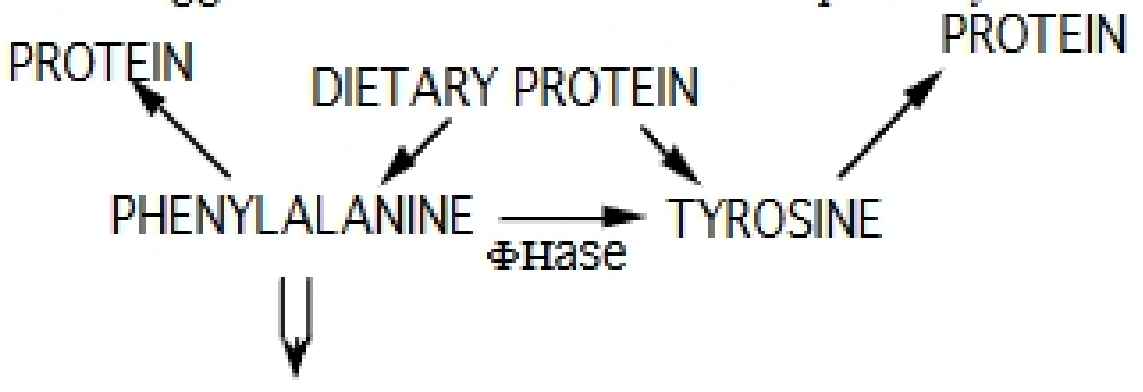
PKU /_ = normal
pku/pku = affected

It is rare, only about 1 in 11,000 live births is affected in the US

In addition to excretion of phenylketones in the urine, there is a much more serious consequence of this disease. If left untreated, the average IQ of homozygous recessives will be 17!. 96% of untreated PKU patients have an IQ less than 50 and 64% have an IQ below 20. Again, the defect can be traced to the presence or absence of a single enzyme, but in this case, the enzyme is usually found only in the liver. The enzyme phenylalanine hydrolase (**Φ Hase**) is required to convert phenylalanine (phe) to tyrosine (tyr), the first step in getting rid of any excess that may be present in the blood. When excess phe accumulates, it can be converted to phenylketones spontaneously, which are then found at high levels in the urine. Whether the high levels in the blood prevent access of other essential amino acids into the developing brain cells, or intrerfere with a pyruvate kinase required for energy in developing brain cells is a point of contention. In any case, the problem develops very rapidly after birth of an affected infant. Before birth, the mother, who, at least in the past, has always been a PKU / pku heterozygote, has the enzyme and her "filtering system" keeps the level of phe at acceptable levels for normal prenatal development. There is a much higher than normal level of phe in the infants blood at birth though, and this has led to the development of a simple, accurate test which is given to all babies very soon after birth. The test is called the Guthrie test. It consists of a dried filter paper impregnated with bacteria (*B. subtilis*) and a toxic analogue of phe called beta-thienylalanine. When a drop of blood that has lots of phe is applied, the bacteria can grow; otherwise they cannot. Blood samples taken soon after birth are collected, and can be tested for a few cents each. Follow up chemical tests are also needed, since in some cases, especially for small babies, since they tend to have a high level of phe that does not persist as it will with PKU patients.

But why would we test babies unless something could be done?

You may be able to suggest some treatments from the pathway involved:



Someone usually suggest giving them the enzyme, which may be possible someday via genetic engineering. However, 1) you can't feed a patient the enzyme since they will digest it, 2) you can't inject it since it will probably be recognized as a foreign