(Personally given to JBH by G. Beadle in N.Y. 12/18/41)

(1)

Memorandum regarding Stanford University project on biochemical genetics of Neurospora.

From work already done by numerous investigators it is clear that the interrelated reactions that make up development and function are in some manner under the control of genes. It is tenable to assume, in fact, that every enzymatically controlled reaction is under the immediate control of a single gene and, conversely, that every gene controls directly some primary reaction. The usual method of studying this control is to try to work out the biochemistry of known genetic characters. A more direct way of accomplishing the same end is to attempt to find out about the genetic control of known biochemical reactions.

A procedure has been worked out for making use of this more direct approach. This involves the use of the ascomycete Neurospora, commonly known as the pink or red bread mold. The principle may be illustrated by a specific

~~Example~~. Suppose we assume that a specific gene controls the reaction that combines thiazole and pyrimidine to make thiamine (vitamin B_1) Since ~~##~~ normal strains of Neurospora grow without B_1, they presumably carry on this reaction (or possibly to not need ~~thiamine~~ B_1, an ~~a~~priori improbable ~~situation~~ alternative). By X-raying, we should be able to induce a mutant unable to carry on this reaction if our assumption is correct. The procedure is to X-ray, establish single spore strains on a medium containing B_1 (so that a mutant that cannot make it will ~~not be lost~~ be enabled to survive). Such strains are then tested for loss of ability to combine ~~thio~~ thiazole and pyrimidine by ~~testing the mutants on~~ testing them on a medium containing thiazole and pyrimidine but no intact B_1 molecules. Inability to grow on such a medium indicates

mutation of a gene concerned with this particular ~~reaction~~ steps in the synthesis of vitamin B_1. Similar procedures can be ~~for~~ developed for other reactions, in fact, tests for mutants for many reaction ~~many~~ can be combined into a single preliminary procedure.

~~Its~~ In terms of experimental procedure the ~~ols~~ scheme followed is made up of The following steps:

1. X-ray before meiosis
2. After meiosis isolate and grow single sexual spores on a "complete" medium made up of:
   - "minimal" medium plus:
     a. Known vitamins
     b. Unknown vitamins
     c. Known amino acids
     d. Unknown amino acids
     e. Known Other substances
     f. Unknown Other substances

   (The source of a to f is a mixture of yeast extract and malt extract)
3. Asexual (one word) transfer of all strains to a "minimal" medium made up of:
   a. Inorganic salts
   b. Biotin (which Neurospora cannot synthesize)
   c. Sucrose
   d. Inorganic nitrogen (NH_4)
4. Select strains that grow on "complete" but not on ~~complete~~ "minimal"

medium. These have obviously lost the ability to synthesize some essential substance present in yeast extract and/or in malt extract. This substance should fall in one of the classes a to f under 2.

5. Systematic tests of mutant strains on
   a. Known vitamins
   b. Known amino acids
   c. Known other substances.

6. If a known substance, work out steps in synthesis that is blocked, and determine inheritance of mutant.

7. If an unknown substance, attempt to isolate this ~~unkn~~ substance in pure form and determine its chemical nature.

Since the discovery of the induced gene mutations in no way depends on the reaction concerned being known, it is evident that in

addition to mutants unable to make substances of known nature, there should be obtained mutants unable to make, and therefore unable to grow without the addition of, unknown substances. In fact evidence for at least three new vitamins of the "B" group has been obtained. In addition evidence for a new basic amino acid present in casein is at hand. Obviously such mutants constitute specific tests for the unknown substances and as such are essential tools in the isolation of such substances.

Mutants for known as well as for unknown substances can be made valuable use of in developing assays for these substances. Thus, there is already at hand, as the

result of this work, potential assays for:

Thiamine (B_1)
Pyridoxine (B_6)
Nicottnic acid (B_4?)
Pantothenic acid
p-Aminobenzoic acid
Three unknown vitamins
Methionine
Several unknown amino acids.

A simple and apparently reliable general assay method has been partially developed (as a function of concentration of material being assayed) using the linear rate of progression of a mycelial frontier in horizontal tube half filled with agar media. This method looks exceedingly promising and if successfully developed will without doubt find wide application in research, nutritional work, etc.

One other ~~application~~ aspect of the project involves the use of the mutants as tools in ~~the culture~~ the study of basic cell physiology. Already the use of the p-aminobenzoic acid deficient mutants has been demonstrated ~~to~~ to be useful in the study of the ~~act~~ action of sulfanilamide and related compounds. Thus we know that in Neurospora sulfanilamide acts by competing with p-aminobenzoic acid rather than by interfering with its synthesis