BIOCHEMICAL GENETICS

There have been two chief biochemical approaches to the study of genetics—through the biochemical study of the effects of gene substitutions, and through a direct attack on the chemical nature of the genetic material itself. Both approaches have been highly successful in recent years, but both went through a rather long period of slow development that was often rather discouraging.

The study of the biochemical effects of genes may be dated from the work of Garrod on alkaptonuria in man. In 1902, he concluded that this condition is an inherited one and that it is due to an alternative pathway in the metabolism of nitrogenous materials, leading to the excretion of homogentisic acid—rather than its further degradation product, urea—in the urine. He consulted with Bateson on the genetic question, and in 1902 the latter discussed the case, giving what appears to be the first suggestion of gene action in terms of "ferments" (enzymes) and of a recessive owing its properties to the absence of a particular "ferment." Later, Garrod (1908) discussed the case in much more detail and concluded that the condition is due to the blocking of a particular enzymatically controlled metabolic reaction, leading to an accumulation and excretion of the substrate (homogentisic acid) normally degraded by this reaction. He deduced the probable earlier compounds produced in this degradation and administered them to alkaptonuric patients. They were degraded to homogentisic acid, thus showing that the mechanism was interrupted at that one point but was still capable of functioning up to that point.

He also drew similar conclusions concerning some other less fully analyzed biochemical defects in man such as albinism, cystinuria, and porphyrinuria.

This work was early recognized as of great importance in the study of the chemistry of metabolism; it illustrated the principles of blocking of specific metabolic pathways, and the resultant accumulation of intermediate products, in intact living organisms. In 1909 the results were dis-

cussed by Bateson in what was the standard general work on genetics—required reading for every geneticist. He pointed out the general similarities between gene action and the action of enzymes, especially in their specificity and their effectiveness in very low concentrations. He concluded that genes often act through the production of enzymes but was doubtful that they could always do so, since he found it difficult to suppose that such a character as brachydactyly could be caused by the addition of an enzyme to the system. He also urged that the enzymes in question should be thought of as gene products, not as the genes themselves.

Bateson was influenced by the work at Cambridge—evidently stimulated by him—on the correlated genetics and biochemistry of the melanins in mammal coat colors (Durham, 1904) and of the anthocyanins in flower colors (Wheldale 1909). Cuénot had discussed the effects of genes influencing the coat colors in mice in terms of chromogens and enzymes in 1902 and 1903. These studies were continued by others (especially by Gortner and by Onslow with melanins and by Scott-Moncrieff with anthocyanins), and led to rather detailed descriptions of the effects of particular genes in biochemical terms, with enzymes regularly assigned major roles.

In 1917 Wright brought together the chemical and genetic data on the melanins in mammals, comparing all the forms on which evidence was available and arriving at a general scheme that rested on the assumption that the known genes produced their effects by conditioning the presence and the specificity of a few enzymes.

There was a widespread reluctance, however, to draw conclusions about direct gene action, since most geneticists were inclined to lay great emphasis on the complexity of development and to conclude that this approach had little chance of throwing light on the direct action of genes. The view that it may sometimes be possible to study fairly direct products of gene action grew out of the studies on the antigens of red blood cells, as described in Chapter 15. This idea soon bore fruit.

It was shown by Morgan and Bridges (1919), in their study of the gynandromorphs of Drosophila, that the sex-linked genes are usually "autonomous"; that is, each separate part of the body develops according to its own genetic composition, with no visible effects on the composition of the rest of the body. This type of analysis has also made use of the twin spots arising from somatic crossing over (Chapter 8). I showed in 1920 that the mutant vermilion is an exception to this rule. In gynandromorphs that are mosaics for vermilion (v) and wild-type (v^+) tissue, it sometimes happens that genetically v tissue develops the v^+ eye color,

evidently through the influence of something it acquires from the v^+ tissue.

The next step in the analysis of this type of phenomenon was made by Caspari (1933), using the meal moth Ephestia. In this form there is a recessive mutant *(a),* that has pale eyes and testes. Caspari made reciprocal transplants of testes between larvae of the two forms (*a* and a^+), using a technique developed by Meisenheimer (1909) for other purposes with other moths. The results of Caspari's experiments were that: (1) *a* testes implanted in a^+ hosts developed darker color, and (2) a^+ testes implanted in *a* hosts not only developed full color but also induced a darkening of the testes and of the eyes of the recipient. Evidently here, again, there was a transfer of something from a^+ tissue to *a* tissue; in more recent terminology, *a* of Ephestia, like *v* of Drosophila, is a *reparable* mutant.

In 1935, Beadle and Ephrussi adapted the transplantation technique to Drosophila, by removing imaginal discs from one larva and injecting them into another larva. These implanted discs persisted and at metamorphosis developed into the parts they would have produced if they had been left in the original donor—though these parts usually lay free in the body cavity and did not replace the corresponding parts of the recipient larva. Through the use of this technique, Ephrussi and Beadle were able to show that the eye discs of *v* and v^+ Drosophila behave like the testes of *a* and a^+ Ephestia; *v* discs implanted in v^+ hosts develop the v^+ color, and the eye color of *v* hosts is modified toward v^+ by the presence of v^+ implants.

A similar study using many other mutant types showed that most of them were autonomous in development, thus confirming the conclusions derived from gynandromorphs. But the recessive eye color cinnabar *(cn)* proved to be reparable, behaving quite like vermilion *(cn^+* and the a^+ of Ephestia were later shown to be mutually substitutable). The two mutants, *v* and *cn,* are alike in phenotype, both lacking one of the two pigments (the brown one) that are present in wild-type eyes. When reciprocal transplants were made between vermilion and cinnabar larvae, it was found that vermilion discs in cinnabar hosts developed wild-type eyes, but cinnabar discs in vermilion hosts failed to develop the brown pigment. That is, *cn* hosts produce the v^+ substance, but *v* hosts do not produce the cn^+ substance.

Beadle and Ephrussi (1936) concluded that the two substances are sequential in the normal metabolic chain, and that the reactions may be represented thus:

$$\text{substrate} \rightarrow v^+ \rightarrow cn^+ \text{ substance}$$
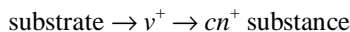
This type of argument has since been applied to many systems and has proved to be one of the most powerful methods in the analysis, through the study of mutant types, of metabolic syntheses in intact organisms.

The study of this system was actively carried on by Ephrussi and co-workers (Khouvine and others), by Beadle and his co-workers (Tatum and others), and by others in several laboratories. These studies, culminating in the identification of the v^+ substance as kynurenine (Butenandt, Weidel, and Becker, 1940) have been described many times—for example by Ephrussi (1942).

The use of Drosophila for this kind of work was stimulated by the existence of a large number of mutant types, whose interaction, it was hoped, could be useful in future studies; but with the discovery that the great majority of these are autonomous it became evident that the transplantation technique was not useful in their analysis.

This realization led Beadle and Tatum to look for a more favorable object. They chose the fungus Neurospora. The techniques for handling this material had been worked out by Dodge and by Lindegren, who had also used it for interesting genetic studies. It had also been shown by Robbins that Neurospora can be grown on a relatively simple synthetic "minimal medium," containing a carbon source, small amounts of inorganic salts, and a single complex organic compound (biotin). On this medium the plant manufactures for itself the other organic substances that it needs—amino acids, vitamins, and other, still more complex, substances such as proteins. Beadle and Tatum reasoned that it should be possible to detect and to study any mutants affecting the ability to synthesize such substances, providing they were reparable. That is to say, if the substance not produced by a mutant could be supplied in the medium and then utilized, growth should occur, and genetic and biochemical studies could be carried out.

The plant has other advantages for this purpose. It is haploid, so that dominance and recessiveness do not complicate the analysis; it usually reproduces asexually by the production of numerous "conidiospores," which make it possible to work with very large numbers of individuals of identical genetic composition and also simplify the detection of large numbers of mutant individuals; sexual reproduction can occur, making possible a detailed genetic analysis of the mutants produced.

In their first paper on the subject, Beadle and Tatum (1941) reported the recovery of three mutant strains after X-ray treatment. These grew normally on a "complete medium" made by adding malt extract and yeast extract to the "minimal medium" described earlier but were (unlike the wild type from which they came) unable to grow on the minimal medium

itself. Further analysis showed that they could be grown on minimal medium supplemented with pyridoxine (vitamin B_6), thiamine (vitamin B_1), or para-aminobenzoic acid, respectively. The type that required pyridoxine was shown to be inherited as a single-gene mutant type—as were the other two and a whole series of additional mutants that were soon reported, in papers quickly following the original account. The techniques were gradually improved in several ways and were developed so that spontaneous mutants (occurring with a very low frequency) could be studied, as well as those induced by X rays or ultraviolet light.

These studies were rapidly extended at Stanford (Beadle, Tatum, Mitchell, Horowitz, Houlahan, D. Bonner, and others) and other laboratories. They led to work with similar methods on other microorganisms, following the discoveries of sexual reproduction or similar phenomena leading to recombination in yeast (Winge, 1935); bacteria (Avery, MacLeod, and McCarty, 1944; Tatum and Lederberg, 1947); and bacteriophage (Hershey, 1946).

These studies have led to great advances in our knowledge of metabolic pathways and in our understanding of the mechanisms of gene recombination and of the ways in which genes act. These studies are outside the scope of this book, but mention must be made of the "one gene–one enzyme" hypothesis, which is now dominant in the study of gene action.

The biochemical approach to the study of the nature of the genetic material goes back to the work of Miescher (1871). He studied the nuclei of pus cells (and, later, of fish sperm cells) and described a substance that he called nuclein. The paper was submitted in 1869, but the editor, Hoppe-Seyler, held it for two years while he repeated some of the observations that had seemed to him to be rather improbable. There followed a series of papers on the subject by Miescher and others. In 1889, Altmann showed that the substance could be split into protein and nucleic acid—the latter being unusual among complex organic compounds in lacking sulfur, but containing much phosphorus. By 1895, Wilson could write:

> Now, chromatin is known to be closely similar to, if not identical with, a substance known as nuclein—which analysis shows to be a tolerably definite compound composed of nucleic acid (a complex organic acid rich in phosphorus) and albumin [protein]. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular chemical compound from parent to offspring.

In the next year he suggested that it is the nucleic acid component that is responsible for heredity.

The existence of two kinds of nucleic acid was shown by Kossel in his

studies of material derived from thymus and from yeast cells. These two types, long known as "thymus nucleic acid" and "yeast nucleic acid," are now known as deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA), respectively. Ascoli (1900) and Levene (1903) showed that both contain adenine, cytosine, and guanine, while the thymine of DNA is replaced by uracil in RNA. Levene also established the presence of deoxyribose and ribose, respectively, as the sugars present.

The early analyses of nucleic acids suggested that the four bases were, in each case, present in equimolar amounts, and the "tetranucleotide" interpretation was developed. According to this view, an essential component of the nucleic acid molecule is a unit composed of one of each of the four bases. Chargaff and his co-workers showed (1950 and later) that in DNA the bases need not be present in equimolar amounts, but that the amount of cytosine does equal that of guanidine, and that of adenine is equal to that of thymine.

With the development of a stain specific for DNA (Feulgen and Rossenbeck, 1924) and, later, of enzymatic methods for distinguishing DNA and RNA, it became possible to study the distribution of both substances in the cell. Feulgen (1937) showed that, in most cells, almost all the DNA is in the nucleus.

Another approach grew out of the studies of Griffith (1928) on the bacterium Pneumococcus. It had been found previously that this organism exists in a number of serologically distinct types (Neufeld and Haendel, 1910; Dochez and Gillespie, 1913, and others, later). The type specificity is due to differences in the mucopolysaccharides in the capsular envelopes of the bacteria, and in culture of the strains *in vitro* it may be lost, leading to "rough" strains that have lost both their type specificity and their pathogenicity. Griffith (1928) used a rough strain that had been derived from a Type II strain, which he injected into mice along with killed individuals of Type III. The mice died, and from their bodies Griffith recovered virulent Type III strains of Pneumococcus. This was the first example of transformation, as the phenomenon came to be called. Dawson and Sia (1931) succeeded in getting it *in vitro* instead of making the mixture in a mouse. The studies were continued, in an effort to isolate the "transforming principle" from the killed virulent strain, and in 1944 it was shown by Avery, MacLeod, and McCarty that this agent is in fact DNA—though the specificity conferred by it and inherited by the descendants of the transformed cells is due to a polysaccharide.

It had been generally supposed that the degree of specificity evidently present in hereditary material could only reside in proteins. Partly because of the tetranucleotide interpretation, it had seemed that the nu-

cleic acids were too simple for this degree of specific diversity. With the breakdown of the tetranucleotide theory, and even more because of the Pneumococcus result, attention turned to DNA. The spectacular results in this field in recent years are not within the scope of this book, since they have been described many times, and are still being very actively developed. I can only mention the solution of the structure of DNA by Watson and Crick (1953) and the work on the RNA code by Nirenberg, Ochoa, and many others.