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Chapter 6

Physiology of Gene
Action in Hybrids

The physiology of gene action in hybrids is not a subject apart from the physiology of gene action in organisms in general. The approach to specific problems of gene action is probably better made in non-hybrid organisms than in hybrids. Hybrids do, however, represent one type of genetic situation which in certain instances is particularly favorable for the study of gene action. Most useful in this respect are those hybrids which exhibit the phenomenon referred to, often rather loosely, as hybrid vigor. The terms hybrid vigor and heterosis often are used synonymously. A more precise usage, and one in accord with the original definitions, refers to the developed superiority of hybrids as hybrid vigor, and to the mechanism by which the superiority is developed as heterosis. By this definition, hybrid vigor is heterosis manifest. Because in studies of growth and development it is often desirable to distinguish clearly between mechanism and end result, this use of the two terms will be followed in this chapter.

Heterosis has been the subject of many experiments and a great deal of speculation on the part of geneticists. The concern has been mostly with the genetic bases of heterosis, and relatively little attention has been given to the physiological mechanisms involved. As a matter of fact, the literature on heterosis mirrors faithfully the changing emphasis in genetics in the last two or three decades. Practically all of the early investigations of heterosis had to do with the comparison of mature characteristics of inbred lines and their vigorous hybrids, and then with attempts to formulate genetic schemes in explanation of the differences. Gradually, the focus of investigation has turned to a study of developmental differences responsible for the hybrid vigor, and more recently to the gene action bases of these developmental differences.

It is a fair hope that from detailed studies of the nature and development of heterosis, much will in time be revealed about specific gene action. Unfortunately, most of the studies up to the present time have been directed to general rather than to specific considerations. It has been necessary to deal in terms of size differences, yield differences, and growth rate differences, until enough of the pattern should appear to indicate what specific physiological considerations are likely to be involved in heterosis. Because we have come only to this point and have proceeded but a little way in an analysis of these specific physiological considerations, this chapter will have to deal more with suggestions of the likely mechanisms than with data from investigations of them.

It is neither possible nor desirable to separate wholly the consideration of the physiological mechanisms of heterosis from the genetic bases. Our main concern will ultimately be with the genes involved and the nature of their action.

The word hybrid has no good, definitive genetic meaning. It can be used with equal propriety to refer to organisms which approach complete heterozygosity or to organisms which are heterozygous for only a small number of genes.

There is at least a rough relationship between the amount of heterosis in a hybrid and the extent of the genetic differences between the parents. Physiological and morphological diversity are dependent both upon the number of allelic differences between organisms and upon the nature of the action of the particular genes among which these allelic differences exist. It is quite possible that organisms differing by only a few genes may be more widely separated in certain characteristics than are organisms differing by many more genes—the actions of which are of less fundamental significance for the control of the developmental pattern.

In our approach to questions of hybrid vigor, we may be concerned with different degrees of hybridity. Consideration of this factor must involve not only the number of genes but also the nature of the action of the particular genes. Nor is this all, for the action of any specific allele is conditioned by the genetic background in which it occurs in a particular individual. Hence, the relations among genes may often be of critical importance.

Of tremendous import, too, are the interactions between the activities of the genes and the environment. In speaking of hybrid vigor, we are generally concerned with such characteristics as size and yield, but these are merely end products of the metabolic processes. Patterns of these metabolic processes are set by the genes, but the processes themselves may be either accelerated, inhibited, or otherwise modified by the effects of environmental factors. Hybrids which are particularly vigorous under certain conditions may show relatively little vigor under other environmental conditions. It is true that the enhanced vigor of hybrids frequently gives to them a wide

range of environmental adaptability. It is equally true that certain hybrids exhibit vigor within only relatively narrow environmental limits. For lack of evidence it must be assumed that the distinction lies in the differences between the patterns of hybridity and in the action of the genes responsible for the hybrid advantages.

Any attempt to explain the genetic basis of heterosis must make initial recognition of one fact. The phenomenon can involve only the recombination of alleles already existing in the population or populations from which the hybrid organisms have been developed; unless, by rare chance, mutation should take place just prior to or just after the actual crossing. We are thus concerned with an interpretation limited to different types of recombinations, and to different kinds of gene action resulting from these recombinations.

GENETIC MECHANISM OF HETEROSIS

Consideration of the characteristics of dominance and heterozygosity has been of primary importance to investigators concerned with interpretation of the genetic mechanism of heterosis. Jones's dominance of linked factors hypothesis (1917) probably is still the most popular explanation of the genetic basis of heterosis.

Dobzhansky (1941) and his co-workers, and many others, have recorded that in most species there has been, in the course of evolution, accumulation of deleterious recessive characters, which when homozygous reduce the efficiency of the organism—but which in the heterozygous condition are without efficiency-reducing effects. This revelation calls for a reshaping of notions regarding the nature of the favorable effects of the dominant alleles, but does not otherwise modify the structure of the explanation. The favorableness of the action of many of the dominant alleles probably is not the result either of directional mutation producing more favorable dominants or of selection tending to eliminate the unfavorable dominants. Instead, it may be due to the accumulation in populations of deleterious recessive mutations. These, if their effects are not too deleterious, often can be piled up in significant numbers.

The piling-up of such deleterious recessives is probably one of the reasons why heterosis is a much more important phenomenon in such a plant as corn than it is, for example, in the tomato. Corn has been handled for hundreds or even thousands of years in a manner that has made possible the accumulation in populations of relatively large numbers of deleterious recessive modifiers. The tomato is more than 90 per cent self-pollinated, and any great accumulation of deleterious modifiers is unlikely. Corn populations characteristically contain thousands of individuals, and wind pollination makes for maintenance of heterozygosity. In tomato, the effective breeding population size approaches one, and deleterious mutations would tend to become

homozygous with sufficient frequency to bring about the elimination of many of them.

As a matter of observation, it would seem that a comparison of the occurrence and degree of heterosis in different species, along with a consideration of the reproductive mechanisms in the various species, supports the proposal that heterosis in many cases is the result of the covering up in the hybrids of deleterious recessive alleles with a consequent return to vigor. The often stated argument that hybrids of corn, for instance, frequently are more vigorous than the original open-pollinated populations from which the inbreds used in their production were derived, has no validity with respect to this situation. In the production of the inbreds there is invariably a reassorting of the alleles of the open-pollinated populations.

It is highly improbable, however, that dominant alleles operating either because of certain inherent favorable characteristics of their own, or simply to prevent the deleterious activity of recessives, present the only genetic basis of heterosis. Dominance is by no means the clear-cut feature described in Gregor Mendel's original paper. The dominance of a particular allele may be conditioned by the environment, or it may depend upon the genetic background in which the allele exists. A completely dominant effect of one allele over another, in the classic sense of our utilization of the word dominance, is by no means universal.

Rather unfortunately the so-called heterozygosity concept of heterosis has usually been introduced as being in opposition to the dominance explanation. Because the concepts of the features of dominance and recessiveness early put them into rigid categories, it has been difficult to postulate how a heterozygous condition with respect to one or more genes could render an organism more vigorous than the homozygous condition, usually of the dominant alleles.

Evidence of significance for the interpretation of the importance of heterozygosity in heterosis has been accumulated slowly. There is now, however, a fairly long list of instances in many different species in which the heterozygous condition for certain alleles is known to be superior to either the homozygous recessive or the homozygous dominant condition (Stubbe and Pirshcle, 1940; Singleton, 1943; Karper, 1930; Robertson, 1932; Robertson and Austin, 1935; Gustafsson, 1938, 1946; Nabours and Kingsley, 1934; Masing, 1938, 1939a, 1939b; Rasmusson, 1927; and Timofeef-Ressovsky, 1940.

The accumulation of data on these cases followed a long period during which all the investigations reported seemed to indicate no marked differences between organisms heterozygous for certain alleles and those with the dominant homozygous condition for these same alleles. At least, in no instance, was there any marked superiority referable to the heterozygous condition. Most of the genes involved in the more recent findings have been

catalogued as having at least moderately deleterious effects in the mutated state. The characteristics controlled by them include: chlorophyll deficiencies, modifications of leaf form and pigmentation, stalk abnormalities, flowering pattern, and time of flowering.

The extent to which the actual nature of the genetic situation has been analyzed varies, but in several of the cases it seems clear that the mutation of a single gene is involved and that the F_1 hybrids are heterozygous only with respect to the alleles at this particular locus. The amount of heterosis manifest also varies greatly. Because of experimental differences, no accurate comparisons can be made, but in some instances the amount of hybrid vigor appears to be nearly comparable to that which occurs in crosses involving large numbers of allelic differences. The situation appears to be one in which a mutation takes place, and the mutated allele is definitely deleterious when homozygous. In individuals heterozygous for the particular gene, there appear none of the deleterious effects. Instead, a definite heterotic effect appears. Dominance is of no apparent importance, and the distinction between the vigorous hybrids and the less vigorous non-hybrids rests upon heterozygosity.

Jones (1944, 1945) has reported several cases of what he has called heterosis resulting from degenerative changes. He first suggested that these cases represented instances of heterosis with a genetic basis in the heterozygosity of certain of the mutated genes. More recently (private communication) Jones has concluded that these cases involve more than single gene differences, and that the results may be explained on the basis of an accumulation of favorable dominant effects.

The case of a *single locus heterosis* reported by Quinby and Karper (1946) involves alleles which do not produce any detectable deleteriousness, but in certain heterozygous combinations produce hybrid vigor comparable in amount to that in commercial hybrid corn. Quinby and Karper have referred the hybrid advantage in this case to a stimulation of meristematic growth in the heterozygous plants.

All of these instances involve specific allelic interactions and not superiority resulting from heterozygosity *per se*—as was postulated by some of the earlier workers concerned with the genetic interpretation of heterosis. These examples contribute to the increasing realization that the phenomenon of dominance is perhaps of less importance with respect to heterosis than has been supposed. There is no *a priori* reason why the interaction of a so-called recessive allele and a so-called dominant allele should not give results different from and metabolically superior to those which are conditioned by either two recessives or two dominants.

This situation bears closely upon the interpretation of heterosis set forth by East in 1936. East postulated that at the loci concerned with the mechanism of heterosis there might be a series of multiple alleles—with the combinations of different alleles giving results metabolically superior to

those determined by the combinations of like alleles, and with no considerations of dominance being involved. In the light of existing evidence it seems a safe assumption that a considerable portion of hybrid vigor is the result of allelic interaction between different alleles at the same locus. Although the evidence as yet is scanty, it is certainly pertinent to suggest that some heterosis may result from the interaction of alleles at different loci, when such alleles are brought into new combinations in the hybrids.

Most of the recent studies of the relation of heterozygosity to heterosis have been concerned with the results of the action of single genes. Such studies have emphasized that heterosis need not have its basis in the action of large numbers of genes but can be, and apparently frequently is, a result of the combining of different alleles of a single gene. Any considerable amount of hybrid vigor resulting from the action of single genes would seem to indicate the involvement either of multiple effects of single genes or of genic action in the control of relatively fundamental metabolic processes. Both are likely probabilities.

The metabolic system of any organism which grows and functions in a satisfactory manner is an exceedingly complicated mechanism with a great number of carefully balanced, interrelated processes. The mutation of any gene which has control over any of the key processes or functions will almost certainly be reflected in a number of processes and activities. For example, if a change in the character of some fundamental enzyme system is involved, either the addition or subtraction of a functional step, or of a substance produced at a particular developmental stage, would be likely to enhance or inhibit a number of important processes in the general metabolism of the organism.

The equilibrium factor in genic action is obviously a consideration of great importance. If a mutation disturbs this equilibrium after it has become fairly well established through selection and elimination processes, the consequences may reduce the organism's vigor. If, in a hybrid, the mutation is then brought together with the original wild type or normal allele, the sum total of the actions of the mutated allele and the original allele may well be such as to exceed that of two copies of the original allele in the production of vigor in the organism.

When we give attention to physiology of gene action in hybrids which are heterotic, we must concern ourselves with all of these considerations including the fact that a single gene, the mutation of which affects some processes in a sufficiently fundamental stage of the organism's formation, may well have a greater end effect than a number of genes whose functions are concerned with more superficial developmental processes.

SEED AND EMBRYO DEVELOPMENT

The literature on heterosis contains a number of discussions concerning the relation between seed and embryo size and heterosis (Kiesselbach, 1926:

Ashby, 1930, 1932, 1937; East, 1936; Sprague, 1936; Copeland, 1940; Murdoch, 1940; Kempton and McLane, 1942; Whaley, 1944, 1950).

Most of the investigations have dealt with mature seed and embryo size. The evidence shows that in many instances hybrid vigor is associated with a high embryo weight. In some cases the initially high-weight embryo is found in a relatively large seed. There is, however, by no means a consistent correlation between either high embryo weight or large seed size and heterosis.

The results of studies on corn inbreds and hybrids in our own laboratory (Whaley, 1950) seem representative of the general findings. Among some ten inbred lines there occurred a great deal of variation from one line to another as to both embryo weight and seed weight. There was somewhat more variation with respect to embryo weight. Among the F_1 hybrids, all of which exhibited considerable vigor under central Texas conditions, there were a few with embryo weights which exceeded those of the larger-embryo parent. For the most part, the embryo weights were intermediate, and in one or two cases they were as low as that of the smaller-embryo parent. The weight of the seed tissues other than the embryo tended to follow that of the pistillate parent, but was generally somewhat higher. Double crosses which had vigorous F_1 hybrids as pistillate parents characteristically had large seeds with what were classified as medium-weight embryos.

The few reports, such as Copeland's (1940), concerning the development of embryos in inbred and hybrid corn, suggest that at the earlier stages of development some hybrid vigor is apparent in the hybrid embryos. The observations of hybrid vigor during early development of embryos and the absence of any size advantage at the time of seed maturity are not necessarily conflicting. In most plants, embryo and seed maturation represent fairly definite stages at which a certain degree of physiological maturity and of structural development has been attained. It is probably to be anticipated that even though certain heterotic hybrids show early embryo development advantages, these advantages may be ironed out by the time the embryo and the seed mature. The size of both the embryo and the other seed tissues is conditioned not only by the genotype of these tissues themselves, but also by the nutritional background furnished them by the plant on which they grow.

It is quite possible that this genotype-to-background relationship is an important consideration in the determination of whether or not hybrid vigor is exhibited in the development of the embryo and seed. The background provided by the pistillate parent might be such as to preclude the development of embryo vigor, even though the embryo genotype were of a definitely heterotic constitution. The fact that hybrid vigor is apparent during certain stages of embryo and seed development may or may not be related to an embryo or seed size advantage at maturity. Because of this, it seems doubtful that embryo or seed size is a reliable measure of hybrid vigor; and that

the rate of development during the embryo and seed maturation period is of any critical importance with respect to the development of hybrid vigor during post-embryonic growth.

EARLY SEEDLING GROWTH AND HETEROSIS

There have been few studies of early postgermination growth in plants in relation to heterosis. It would seem that the usual failure to find higher growth rates during the grand period of growth, or longer continued growth periods in heterotic hybrids, would suggest that the answer to the development of hybrid vigor lies for the most part in the early postgermination growth stages. The work of Ashby and his co-workers (Ashby, 1930, 1932, 1936; Hatcher, 1939, 1940; Luckwill, 1937, 1939) emphasized that the hybrid advantage in their materials was either present in the resting embryo or became manifest in early postgermination growth. Its development was definitely not a characteristic of the later growth phases. This observation has now been made for many cases of hybrid vigor (Whaley, 1950). There are some instances in which hybrid vigor seems to be the result of longer-continued growth on the part of the hybrid. These probably have a different explanation from the majority of cases.

We have been concerned lately in our own laboratory with an analysis of the early postgermination growth of corn inbreds and single and double cross hybrids (Whaley, 1950). Studies of growth during the first ten to twelve days after germination have revealed that the hybrid advantage is largely the result of the heterotic hybrid plants reaching a high growth rate earlier than do the inbreds. Almost without exception, the development of the hybrid advantage takes place very rapidly in the early stages of germination and growth. Rarely have we seen evidence of the hybrids having higher growth rates during any later part of the developmental cycle. Neither are the hybrid growth periods extended appreciably beyond those of the inbreds. In most instances the hybrids mature somewhat more rapidly than the inbreds—a fact of common observation among plant breeders.

Since the attainment of the maximum growth rate takes place more quickly during the early stages of development, the hybrids do have a longer maximum growth rate period. During this period the early advantage is compounded, to give a considerably greater maturity advantage. When both the inbred lines and the hybrids used in our studies are considered, it is apparent that the rapid attainment of high early growth rates is correlated with relatively low embryo weights. This apparent higher efficiency of small embryos and its importance in relation to hybrid vigor requires further study.

On the basis of the data at hand one can suggest that the hybrid advantage lies in the more rapid unfolding of certain metabolic processes, a suggestion which receives support from the recorded studies of later growth.

LATER GROWTH AND HETEROSIS

It is unfortunate that most studies of the physiology of heterosis have been confined to the later growth period, and consequently do not include that part of the growth cycle during which the important differences seem to be developed. Nonetheless, we can learn much from these studies of later growth as to the nature of the physiological differences which may furnish bases for the development of hybrid vigor.

The early experiments on physiological differences between inbreds and hybrids were concerned mostly with the responses of the inbreds and the hybrids to different soil conditions. A few examples will serve to indicate the type of investigation and the character of the results. Hoffer (1926) determined the amounts of the constituents of the ash of heterotic hybrid corn to be generally intermediate between those of the parental types. He noted that iron and aluminum were present in the ash of the hybrids in smaller amounts than in the inbreds. His studies showed that although there were marked differences in the absorption of iron and aluminum in different soil types the vigorous hybrids tended to absorb less of both these elements than the less vigorous inbred lines.

In the same year Kiesselbach (1926) reported distinct differences in water requirements between selfed lines of corn and their heterotic F_1 hybrids. The low productivity inbreds had much higher water requirements than the vigorous F_1 hybrids, when water requirements were calculated on the basis of either water absorbed per gram of ear corn or water absorbed per gram of total dry matter. Barley inbreds and heterotic barley hybrids were shown by Gregory and Crowther (1928, 1931) to make distinctly different responses to various levels of available minerals. These investigators postulated that heterosis in barley might be directly related to differences in the ability of the hybrids and the inbreds to use certain nutrients. This suggestion has had a fairly adequate test, particularly with reference to nitrogen and phosphorus nutrition.

The work of DeTurk et al. (1933), Smith (1934), Lyness (1936), Harvey (1939), Burkholder and McVeigh (1940), and Rabideau et al. (1950), has provided a fairly adequate picture of the relation of phosphorus and nitrogen nutrition to the development of hybrid vigor. Smith demonstrated distinct differences among inbred corn lines with respect to phosphorus nutrition, noting that these differences were most apparent when the phosphorus supply was limited. He postulated that the higher phosphate utilization efficiency of the hybrids might be referred, at least in part, to the dominant inheritance in them of a much branched root system. Later studies have shown that the root growth pattern is certainly important in relation to heterosis.

Smith noted particularly that when inbred lines were inefficient in the utilization of phosphorus or nitrogen, crossing them failed to produce hybrids showing any evidence of physiological stimulation resulting in the more

effective use of these elements. Lyness (1936) studied heterotic F_1 hybrids resulting from crosses between a low phosphorus-absorbing capacity inbred and a high phosphorus-absorbing capacity inbred. He found the heterotic F_1 plants to have high phosphorus-absorbing capacity. These results suggested that phosphorus-absorbing capacity in corn, in some instances at least, acts genetically as a dominant factor. Lyness also noted the relationship between high phosphorus absorption and the extent of root development. He supposed that the extent of root development might be responsible for varietal differences in phosphorus absorption, a supposition which is supported by later studies. The work of DeTurk *et al.* (1933) suggested that more than simply phosphorus-absorbing capacity is involved. This work revealed that the actual phosphorus content patterns of two F_1 hybrids of corn were quite different. By estimating the amount of phosphorus in various chemical fractions, De Turk and his coworkers were able to demonstrate marked phosphorus pattern differences and to associate these pattern differences with various phosphate fertilizer treatments.

In our laboratory we have made a study of the phosphorus-absorbing efficiency of corn inbreds and hybrids, and have attempted to correlate the findings of this study with developmental changes in the vascular system and with general growth (Whaley *et al.*, 1950; Heimsch *et al.*, 1950; Rabideau *et al.*, 1950). The data indicate that heterotic hybrids definitely absorb more radioactive phosphorus than their inbred parents. This advantage in absorption on the part of the hybrid is associated with more rapid early development, with earlier attainment of maturity, and with certain features of vascular organization. The greater absorption can be referred at least in part to better early development of the root system in the hybrids, and to a generally higher level of metabolic activity which presumably creates a greater phosphorus demand. The greater absorption of phosphorus by the hybrids is certainly one of the factors which compounds the heterotic effects, but it seems doubtful that it is a primary factor in the development of hybrid vigor.

Harvey's (1939) studies of nitrogen metabolism among inbreds and hybrids of both corn and tomato showed differences from one line to another with respect to the ability to use nitrate and ammonium nitrogen. The experiments were of such a nature as to make it clear that such differences in nutritional responses were results of differences in genetic constitution.The behavior of hybrids produced from the inbreds reflected a combination of the characteristics of the inbreds. Significantly, Harvey's study revealed that not only did differences exist among his inbreds and hybrids with respect to the ability to use different types of nitrogen, but that there were distinct genetic differences in the responses of the plants to various levels of nitrogen availability.

Somewhat similar differential responses to potassium availability were

revealed by Harvey's studies on tomato inbreds and hybrids. Burkholder and McVeigh (1940) have also noted differences in responses of corn inbreds and hybrids to various levels of available nitrogen. These investigators corre-lated apical meristematic development, and the differentiation of the vascu-lar system with the level of nitrogen nutrition, and the efficiency of different lines and hybrids in utilizing the available nitrogen. Their results indicate that hybrid vigor, involving superiority in the production of dry matter and the differentiation of organs, was not correlated with greater growth and development of the vascular system.

There definitely are vascular organization differences between the heterot-ic hybrids and the inbreds in the material we have studied. These vascular organization differences seem not to be the result of differences in mineral absorption and distribution, but rather to be one of the factors responsible for the differences in absorption and distribution. All the evidence seems to indicate that the greater absorption of minerals by heterotic hybrids can be referred to better developed root systems in the hybrids, probably also to the presence of more efficient transport systems, and to a generally higher level of metabolic activity.

Recently we have undertaken a rather extensive analysis of both the morphological and physiological characteristics of a tomato cross in which there is marked heterosis. We have found no significant differences between the inbreds and the hybrids as to total phosphorus content of the leaves, stems, or roots. There is some suggestion that the phosphorus content of the organs of the hybrids reaches a higher level earlier in growth than it does in the inbreds. Neither do the hybrid plants have any consistent advantage with respect to nitrogen content.

Analyses of the starch content of the leaves and stems suggest that the hybrid plants may have a slightly higher starch content than the inbreds during the early growth stages. In terms of average figures over the whole growth period, however, there are no marked differences between the in-breds and the hybrids. The same appears to be true of the sugar content. The hybrids have a somewhat higher sugar content, at least in the leaves, early in development. During the greater part of the growth cycle the hy-brids do not have significantly more sugar than the inbreds. The only clear difference found between the inbreds and the hybrids is in the catalase ac-tivity of the shoot tips, the hybrids having an appreciably greater index of catalase activity than either of the inbred parents. The catalase activity differences are associated with much more active meristematic growth in the hybrid plants.

THE ROLE OF SPECIFIC SUBSTANCES IN HETEROSIS

Evidence for another sort of physiological differences possibly involved in heterosis is furnished by the work of Robbins (1940, 1941a) in assaying the

growth-promoting activities of extracts from inbred and hybrid corn grains. Robbins' evidence indicates that a substance or substances, which he has designated as factor Z, may be synthesized in greater amounts by the hybrids than by the inbreds. He has stated that factor Z can be fractionated into Z_1, which is hypoxanthine; and Z_2, a still unidentified fraction. Robbins' work suggests that among the advantages possessed by heterotic hybrids may be the ability to synthesize certain growth substances which the inbreds either cannot synthesize or cannot synthesize as well.

Further evidence of a slightly different nature is provided by the root culture work of Robbins (1941b) and of Whaley and Long (1944). Robbins used cultures of a strain of *Lycopersicon pimpinellifolium* Mill., a strain of *L. esculentum* Mill., and their F_1 hybrid, in solutions supplemented by thiamin, thiamin and pyridoxine, or thiamin, pyridoxine, and nicotinamide. Robbins found that the F_1 roots grew much more rapidly and produced more dry matter than those of either parental line. He was able to show further that one parental line made a greater response to the presence of pyridoxine than did the other, while the roots of the second parental line made a greater response to nicotinamide than those of the first. This suggests the combination of complementary factors from the parents in the hybrid. Whaley and Long (1944) obtained essentially the same results with a cross involving two inbred lines of *L. esculentum*.

In the University of Texas tissue and organ culture laboratory, we have been exploring certain aspects of this problem. While the results are not sufficiently complete for publication, some facts are already clear. Among the roots of many inbred lines of tomatoes which we have been culturing, there are marked differences in growth responses associated with the availability or non-availability of thiamin, pyridoxine, niacin, and certain other substances. These differences appear definitely to be inherited and they can be studied in either the inbred lines or hybrids.

It is still too early to say what the inheritance pattern is, but consideration can be given to some aspects of the growth response patterns. One of the most significant revelations is that the responses of most of the roots to a specific substance are conditioned not only by the availability of that substance, but by the availability of the other substances and by the general composition of the culture medium. Heterosis in tomato root cultures is, like heterosis in whole plants, definitely relative, and conditioned, not only by the environment, but, with respect to any specific gene action, by the background of other gene actions taking place in the developing organism.

Heterosis in tomato root cultures is definitely related to the inheritance of the capacity to synthesize or utilize such substances as thiamin, pyridoxine, and niacin. This is not to suggest that heterosis in whole plants of tomato may have its basis in the genetic recombination of factors concerned in the

control of thiamin, pyridoxine, or niacin metabolism. In intact plants, it is likely that the green parts supply these substances to their own tissues and to the roots, in amounts satisfactory for growth and development. The root tissue responses, however, are definitely heterotic in certain instances, and these mechanisms merit examination.

It seems pertinent to explore the role of these B vitamins in growth and development. Thiamin appears to be a metabolic requirement for all types of cells. Its metabolic activity apparently revolves around a role in enzyme systems. Thiamin pyrophosphate is the co-enzyme of the enzyme pyruvate carboxylase (Lohmann and Schuster, 1937). The enzyme carboxylase occurs in many plant tissues. The possible biochemical basis of thiamin action in plants has been set forth in some detail by Bonner and Wildman (1946), Vennesland and Felsher (1946), and Bonner and Bonner (1948). It is assumed that thiamin represents a step in the development of co-carboxylase which is active in one or more of the decarboxylating enzyme systems of the respiratory mechanism.

Pyridoxine also has an enzymatic role, apparently being important for its conversion to pyridoxal phosphate, which is a co-enzyme of one or more of the reactions in the nitrogen metabolism of the plant (Bonner and Bonner, 1948). As a co-enzyme active in nitrogen metabolism reactions, pyridoxine may be of extreme importance in amino acid-protein building, and hence active in conditioning fundamental growth activities.

Similarly, niacin activity is enzymatic in character. Niacin appears to be involved as a constituent of the nucleotide cozymase, and possibly of triphosphopyridine nucleotide. Cozymase is a co-enzyme for a whole series of dehydrogenase enzymes, including alcohol dehydrogenase, malic dehydrogenase, and glutamic dehydrogenase (Bonner and Bonner, 1948).

The genetic background of thiamin, pyridoxine, and niacin metabolism is thus a genetic background concerned with basic components of the plant's enzyme systems. Heterosis, which rests upon recombinations concerned with thiamin, pyridoxine, or niacin metabolism, quite obviously rests upon recombinations which are concerned with the acceleration, inhibition, or blocking of specific stages or developed substances in the basic enzyme system.

A considerable amount of supporting evidence for the involvement of such fundamental enzyme and other growth substance activities in the development of heterosis has been coming for some time from the work on Neurospora. In many heterocaryons of Neurospora, increased growth responses directly suggestive of heterosis have been observed. In a number of instances (Beadle and Coonradt, 1944), the growth responses depend upon the two types of nuclei in the heterocaryon—each carrying wild type alleles of deleterious mutant genes carried by the other nucleus. Such instances represent essentially the same situation as the recombination of favorable dominant alleles in normally diploid organisms.

In one case reported by Emerson (1948) a different situation obtains. A mutant strain of Neurospora which requires sulfonamides for growth at certain temperatures will grow satisfactorily in the absence of sulfonamides, provided that the concentration of available p-aminobenzoic acid is held at a particular level. Either higher or lower concentrations of p-aminobenzoic acid result in growth inhibitions. Emerson has made heterocaryons between a mutant strain carrying the sulfonamide-requiring gene (*sfo*) and a gene which prevents the synthesis of p-aminobenzoic acid (*pab*), and a strain carrying *sfo* and the wild type allele (+) of *pab*. The resultant heterocaryons grow vigorously on the minimal medium (without sulfonamides), whereas strains carrying *sfo* and *pab*, or *sfo* and +, make no appreciable growth on the minimal medium. Emerson's explanation of the growth of the heterocaryons is that it results from a balance between the production of p-aminobenzoic acid by one of the types of nuclei and the absence of production of p-aminobenzoic acid by the other type of nucleus; so that the total production of p-aminobenzoic acid is sufficient for growth but still within the range tolerated by strains carrying *sfo*. Heterosis-like effects of this sort are suggestive of the instances of heterosis related to the heterozygosity of particular genes in diploid organisms.

We thus have in Neurospora, heterosis-like effects assignable both to a recombination of dominant alleles basis and to a heterozygosity basis. More important for this discussion is the fact that these instances are all concerned with facilitation in the hybrid of the production or utilization of substances which are components of the basic enzyme or other growth substance pattern of the organisms.

Various investigations of heterosis in Drosophila, while for the most part not concerned with specific growth substances, have nonetheless assigned manifestation of heterosis to a background in the fundamental biochemical activities of the organisms. Inasmuch as these investigations are discussed in detail in another chapter, they will not be treated here.

THE PHYSIOLOGICAL BASIS OF HETEROSIS

From consideration of the pertinent data, a definite pattern emerges. This associates the development of heterosis with the ability of the hybrid to synthesize or to utilize one or several specific substances involved in the fundamental growth processes of the organisms. Nutritional factors, water absorption factors, and the other more gross considerations with which investigators have been particularly concerned seem to be secondary factors— perhaps responsible for compounding the heterotic effects but probably not responsible for their initial development. Much of the evidence agrees with the assumption that the primary heterotic effect is concerned with growth substances whose predominant activity is registered in the early part of the developmental cycle; in plants, especially in early postgermination

growth. Into this category fall the enzymes, the auxins, and the other "physiological key" substances.

Many heterotic hybrid plants seem to gain their advantage within the first few hours after germination. This advantage may not be shown as statistically significant until it has been further heightened by subsequent growth. The primary growth activities during this period are those involved in the unfolding of the enzymatic pattern; the mobilization, transformation, and utilization of stored materials, and the building up of active protoplasmic synthesis. It seems definitely to be here that the hybrid advantage lies. By the time growth is well under way, the hybrid advantage is already well developed.

Structural differences between inbreds and heterotic hybrids shown by the studies of Burkholder and McVeigh (1940), Weaver (1946), and the members of our laboratory (Whaley *et al.*, 1950; Heimsch *et al.*, 1950; Rabideau *et al.*, 1950) are apparently to be regarded as results of heterosis rather than as causal factors. The evidence suggests that heterosis is concerned primarily with growth processes and that differentiation activities are most likely involved secondarily rather than primarily. What seems to be indicated is the assignment of the physiological basis of heterosis to the activity of one or more of the so-called physiologically active substances involved in early growth.

Much of the apparent hybrid vigor is assignable to these activities only in a secondary fashion. Once the advantage of a larger number of growing centers or of heightened meristematic activity is established, the greater availability of nutrients, the greater amount of protoplasm involved in further protoplasm building, and other general advantages tend to increase the initial differences. To the general evidence in favor of this supposition can be added the specific evidence of the few cases in which the physiological action of particular alleles is known. Where these alleles in combination are responsible for heterosis, they have—when studied in sufficient detail—invariably been shown to be alleles whose action involves basic enzyme or other growth substance activity.

If we are to make significant headway in understanding the physiological mechanism of heterosis, we shall have to concentrate on a detailed study of the developmental physiology of early growth. Much of the general knowledge we already have can contribute toward this understanding if we translate it into terms signifying that when we speak of quantitative differences—size, yield, or of rate differences—we are really concerned with differences in the level of metabolism. We must recognize that these differences in the level of metabolism are bound to vary against different environmental backgrounds, and where the particular genes involved are associated with different genetic backgrounds.

Our approach to the heterosis problem has been complicated by common

insistence upon attempts to find a single genetic mechanism. It has suffered, too, from failure to recognize that between the gene and the final mature organism there lies a system of developmental processes of great complexity. The complexity of this system is formidable but it surely can be analyzed, at least with respect to its most significant features, if it is taken part by part.

SUMMARY

The evidence relating to heterosis suggests that the phenomenon is to be explained genetically in terms of various recombination effects. In some cases, dominance is the important consideration, while in other cases, hetero-zygosity must be considered. In any event, it is the resulting specific gene action which lies at the basis of the physiological advantage or advantages which give rise to hybrid vigor. One or many genes may be involved. Con-siderations of genetic balance and genotype-environment balance are im-portant. Probably most cases of heterosis are to be explained physiologically in terms of differences in the more fundamental aspects of the metabolic pat-tern, particularly those concerned with enzyme, auxin, and other growth substance activity in plants and with enzyme and hormonal activities in animals.

To clarify the mechanism further, studies must be concerned primarily with the genetics and physiology of early development. We have been con-cerned with mature characteristics of size and yield, with the inheritance of so-called quantitative genes, and with analyses by the classic methods of genetics. These studies have brought us close enough to an understanding of the phenomenon of heterosis to indicate that its further analysis by techniques now at hand will uncover facts of tremendous importance for genetics, physiology, and other studies of development, some of them con-siderably afield from heterosis itself.