SYMPOSIUM ON APPLICATION OF GENETICS TO THE STUDY OF DISEASE PHYSIOLOGY

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The Use of Near-Isogenic Lines in Biochemical Studies of the Resistance of Wheat to Stem Rust

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This paper will attempt a heuristic examination, in the light of current theories about the nature of plant disease resistance, of some results obtained with near-isogenic lines of wheat resistant or susceptible to *Puccinia graminis tritici*, race 56. These results have caused the writer to re-examine some articles of faith implicit in his thinking from graduate school years. Re-examination of these articles of faith required some substitute modes of thought which, although speculative, are not necessarily new. Consideration of such substitute modes of thought may be useful at this stage in the development of knowledge concerning the nature of plant disease resistance. A few comments appear necessary in order to place the results, and the speculations derived from them, in perspective. Previous studies in our laboratory have attempted to arrive at a better understanding of the physiology and biochemistry of host-parasite interactions that clearly are classified as susceptible. To some respected colleagues, these studies often seemed to be concerned with events which occur too late during the infection to come to grips with the crucial problem of parasite establishment (e.g., resistance or susceptibility), but there are three major considerations underlying this approach.

Susceptibility is the condition of host plants that the science of plant pathology attempts to alleviate. Studies of the biochemical changes during susceptible interactions may provide opportunities to describe new methods to prevent, alter, or tolerate the susceptible condition. Second, susceptibility is under the same general laws of genetic governance as is resistance. The final phenotypic expression of susceptibility is determined by a sequence of biochemical events in host and parasite which are initiated with the first contact of parasite and host cells. Clarification of the events in susceptibility may be a necessary prelude to understanding resistance through the identification of essential biochemical steps subject to natural abortion (resistance) or to induced abortion (via applied chemicals). For rust diseases, a third and most important consideration

has been the lack of suitable genetic materials for direct comparison of the biochemical sequence which occurs in resistant and susceptible interactions.

The genetic composition of the pathogenic race of rust fungus poses no special problem, since vegetative, asexual propogation provides a single biotype carrying the necessary genetic information for virulence. With the host, however, it had been necessary until recently to compare varieties with a diverse, and largely unknown, genetic background such as, for example, the tetraploid, Khapli, and the hexaploid, Little Club wheats. To a geneticist, the possible experimental pitfalls are obvious from his observation of effects caused by gene substitutions or gene dosages. The pitfalls should be as obvious to the physiologist or biochemist. Total metabolic activity of cells is under genetic control conditioned by environment. Disease itself changes the cellular environment, even for cells not invaded directly. With certain genomes, although not with others, the changes due to disease could trigger, reproducibly, certain identifiable biochemical events, but events that may be spurious with respect to the establishment of compatibility or incompatibility. In terms of classical metabolism, spurious events may arise simultaneously with the biochemical changes resulting in resistance or susceptibility through linkage by common metabolic intermediates, or they may arise sequentially after resistance or susceptibility has been established.

The identification of single genetic loci responsible for rust reaction and the subsequent development of near-isogenic lines carrying either the dominant or recessive allele at a specific locus provide biological systems where such spurious events should be minimized. A number of paired lines, with different genetic loci, and in varying degrees of near-isogenicity, are available for experimental purposes (30). The utility of near-isogenic lines for physiological studies has been suggested in the form of a "quadratic check" in which the four combinations of single genetic factors for host

reaction (alleles for resistance and susceptibility) and pathogen activity (alleles for avirulence and virulence) can be compared (35). An examination of current concepts of disease resistance leads to the conclusion, however, that a "quadratic check", by itself, may not provide rigorous proof of the operation of those biochemical reactions which cause resistance.

Current concepts of disease resistance.—In most of the research approaches to host-parasite interactions, there appears to be among pathologists and plant breeders the implicit notion of resistance as a positive, active, and/or induced characteristic of the host. A glance only at the number of published reviews reveals the acceptance of this view and the scope of efforts to identify antipathogenic chemical factors induced actively by resistant hosts during the process of invasion.

Undoubtedly, active induced resistance is a natural phenomenon but, with full acceptance of the risk inherent in any generalization, it can be argued that there is no single instance where proof is complete for the precise role in disease resistance of any *chemically defined* host component produced during invasion. In examining the literature, it is possible to point to specific deficiencies in individual reports, but the following considerations generally are germane.

1) *Quantitative aspects.*—Despite modern quantitative sophistication, it is still difficult to establish the actual concentrations of suspected antipathogenic compounds at the sites where they act. Tissue concentrations often are measured after extraction with organic solvents because the compounds are sparingly soluble in water. With some of the better publicized compounds of an aromatic nature, it is even necessary to have special solubilization procedures in order to establish ED_{50} values in vitro. The question of water solubility is not an idle one because it bears on the probability of contact of a chemical with an invading pathogen. It is becoming increasingly clear that many secondary plant chemical constituents, such as those of the aromatic or phenolic classes, exist in specialized cells or cellular organelles (perhaps owing to solubility properties), and thus may be sequestered from an invading organism. High, induced concentrations of such compounds in tissue do not necessarily mean effective concentrations for toxicity.

Even with compounds soluble in water at inhibitory dosages, the effective concentrations are not particularly noteworthy for toxicants (10^{-3} to 10^{-4} M), nor do they possess any unusual specificity as toxicants. It has been argued that specificity in host-parasite interactions might be achieved in a two way interaction: (i) the extent of host production of toxicant in response to an organism and (ii) differential toxicity of the compound toward that organism. In the author's view, rigorous quantitative evidence in support of such a picture is quite limited. A serious drawback, rarely mentioned, that handicaps interpretation of many studies of disease resistance is the almost complete absence of quantitative parameters for resistance itself (not chemicals found in resistance) in terms of pathogen development,

number of host cells affected, or degree of host damage. Usually, an arbitrary scale of disease reaction is inherited from other studies originally designed for quite different purposes. If significance is to be attached to determinations of microgram quantities of chemicals found in incompatible reactions, we need more precise definitions (preferably by an objective assay) of the biological phenomenon under scrutiny.

2) *Chronological aspects*—Even if one accepts the potential of some of the identified chemicals to function in disease resistance, a substantial question still remains as to whether such a compound is produced at the proper site in sufficient concentration early enough to account for resistance. Because of the careful development of extensive data by Tomiyama (48, 49) and his colleagues, resistance to late blight in potato well illustrates some of the problems. In vitro, rishitin (26), after appropriate solubilization techniques, is toxic to all tested races of *Phytophthora infestans* at approximately 10^{-4} M. In some cases, an equivalent concentration is not attained in tissue until 48 or 72 hr after inoculation (26). Rishitin was not detected until at least 12 hr (38). Yet, from other studies, resistance as manifested by necrosis is evident no later than 2 to 3 hr after inoculation (49), and as early as 30 min (48).

3) *Experimental verification.*—Most of the evidence in support of induced antipathogenic compounds in resistance can be considered, for lack of better descriptions, as "associative" or "correlative" proofs. A compound or process consistently is observed to be at a much higher level with resistant reactions than that observed with susceptible reactions. In view of the uncertainties cited above, it would be desirable to alter either the biological reaction or the chemical reaction, and then to chart the consequence of such alteration either for the concentration of the chemical or to resistance. Two general types of experiments toward this goal have been tried. Alterations of the environment, particularly temperature, do alter resistance, but suffer experimentally from the fact that, with one exception (33), the alteration has been, or must be, carried out prior to the expression of resistance in the invaded host. Prior treatments do not cause alterations in resistance; the host may be, in fact, susceptible or resistant from the moment of inoculation. With experiments of the "challenge" type, the period of time between sequential inoculations of avirulent and virulent pathogens is sufficiently long (usually 48 hr) so that additional factors, other than the postulated one, may condition development of the second and more virulent pathogen. The technical uncertainties in these types of experiments are formidable (14).

Choice of near-isogenic experimental material.—The above considerations limit the possibility that a simple quadratic check with near-isogenic lines of both host and pathogen will identify, with certainty, those biochemical reactions which cause resistance. Although of great utility in reducing spurious chemical change, the key aspects of

physiological chronology and experimental verification would remain. Fortunately, however, foresight in selection of the genetic factors for resistance led to the inclusion of the *Sr6* gene in the program for production of near-isogenic lines (30). The resistant interaction controlled by the dominant *Sr6* allele is temperature-sensitive. When grown at 20 C and inoculated with race 56, the plants exhibit infection type 0; but at 24-25 C, the infection type for isolated pustules is 4. More important, disease reaction when measured as infection type is reversible, and this confers two experimental advantages, although both are still subject to some uncertainties.

Reciprocal transfers from high or low temperatures at various times after inoculation have suggested that the mechanisms determining compatibility or incompatibility become effective somewhere between the 60th and 80th hr. Still relatively imprecise, perhaps owing to differences in the environment and the experimental protocol used by individual investigators, this observation permits judgments on the chronology of those chemical reactions that are of importance for resistance. Equally important, the reversibility of disease reaction by temperature permits a "challenge" experiment, but by a single inoculation with a single strain of pathogen. Furthermore, the challenge can be carried out either from high to low, or low to high, in terms of infection type and at stages either before or after visible symptoms of resistance or susceptibility appear (2, 41).

Changes in respiration and phenolic compounds with Sr6 alleles.—One of the lines of evidence in accord with the concept of induced disease resistance were observations that oxidative processes of various types appeared to be more active in the early stages of infection in resistant host-parasite combinations than they did in compatible combinations (1). Leaves of Khapli wheat infected with race 15B (infection type 1) apparently showed a sharper and more early rise in respiration rates than did Little Club wheat (37). Antonelli & Daly (2) did not observe such differences when near-isogenic lines with the *Sr6* or *sr6* alleles were compared at 20 C. Until the 5th day after inoculation, the increases in respiration were parallel; then the resistant interaction showed a leveling in rate, whereas the susceptible combination continued to increase during the process of sporulation. The results are in circumstantial agreement with a suggestion that the early increases (20-30%) are owing to the host, whereas subsequent increases, accompanied by a drop in C_6/C_1 ratio (10), occur in the parasite because of the energetic and biosynthetic demands of spore production.

A second unexpected finding arose during comparisons of the content of phenolic compounds in leaves of *Sr6* and *sr6* lines. Tissue concentrations of phenolic compounds were reported to be higher in uninfected wheats, such as Khapli, which exhibit resistance to an above-average number of races of *Puccinia graminis tritici* (27). Furthermore, synthesis of phenolics appeared to occur more rapidly and to a greater extent with resistant interactions of individual varieties.

A detailed study, reported only partially (40), of phenolic compounds extracted and measured in several ways failed to show changes in concentration during infection of lines carrying either the *Sr6* or the *sr6* allele. Other workers employing the same host-parasite combinations earlier reported somewhat greater, but not extensive, incorporation of phenolic precursors into certain chemical fractions with resistant, infected plants, but not with susceptible rust reactions (19, 34). Subsequently, two hydroxyputrescine derivatives of phenolic acids were reported to occur in the infected *Sr6* line at 20 C but not in the *sr6* lines at that temperature (47). As later indicated (36), it does not appear that the derivatives are responsible for resistance because they are also synthesized during susceptible interactions at 25 C with both the *Sr6* and *sr6* alleles, as well as during infection with other wheat pathogens at 20 C.

The obvious inconsistencies in both respiratory patterns and concentrations of phenolics described above could be owing to several factors. The results of the Canadian workers (36) suggested that various environmental stresses, as well as light regime, cause the appearance of the two putrescine derivatives discussed above. It is well known that aromatic biosynthesis generally is influenced by environment, especially light. It is possible that the phenolic response of both susceptible and resistant wheats to infection may be due to spurious events arising from a genetic factor capable of expression with certain environments. There may be, in many varieties, an inherent capacity for phenolic biosynthesis (for example, a full complement of necessary enzymes) under certain environmental conditions so that phenolic metabolism could be triggered nonspecifically by invasion. With near-isogenic lines having Chinese Spring background, a significant capacity for phenolic biosynthesis does not appear to exist under conditions of relatively low light intensity and at a temperature of 20 C (40).

Changes in indoleacetic acid (IAA) decarboxylation and peroxidase activities.—Another phenomenon where differences apparently could be observed during initial stages of infection of susceptible Little Club and resistant Khapli wheat was that of IAA decarboxylation. Shaw & Hawkins (43) reported patterns opposite to those for respiratory changes: an initially more abrupt and higher peak of activity for IAA degradation in susceptible combinations than for the degradation of exogenous IAA in resistant interaction. For several reasons, this observation had particular importance. It had been established that IAA increases in several species as a consequence of rust disease. Furthermore, IAA was reported to lower C_6/C_1 ratios in healthy wheat leaves (44). These phenomena could be related by assuming that, in susceptible reactions, IAA could accumulate in the early stages of infection (12), causing the host to shift to different metabolic pathways conducive to parasite development. In resistant combinations, however, higher rates of IAA

degradation would prevent such alteration. Other studies with susceptible Little Club wheat (11) showed that the decarboxylation of IAA during the first few days after infection is highly variable and affected by light. Furthermore (13), IAA alone did not induce any drastic change in either rates or pathways of respiration.

When sufficient supplies of seed of near-isogenic lines became available, comparative studies at 20 C did show consistent, large increases in rates of IAA decarboxylation in resistant, infected *Sr6* leaves, but not as early as first suggested (43). The increases started at the time (usually 3 days) when temperature transfer experiments suggest that the cellular factors in resistance become evident (2). The increased rate was maintained for as long as 17 days after inoculation, even on leaves with but few visible signs of infection. Decarboxylation of IAA in leaves of *sr6* plants increased somewhat during the first days of infection and then declined, as with susceptible Little Club wheat (11). At sporulation (usually day 6), the rates for infections of type 3 or type 4 were below those of healthy control tissue, whereas the rate in the incompatible combination was 4 to 10 times that of the control.

The precise cellular mechanisms responsible for IAA decarboxylation still are unknown and controversial. Peroxidase, with monophenols as cofactors, can decarboxylate IAA in vitro. Diphenolic compounds inhibit the oxidase. Since phenolic components apparently were not changed appreciably, qualitatively or quantitatively, during infection (40), studies of peroxidase levels were initiated (41). The patterns observed in resistant and susceptible reactions were similar to those for IAA decarboxylation and also to the peroxidase patterns reported by other workers on rust diseases while these studies were underway. Although additional proof is desirable, it seems reasonable that the IAA decarboxylation lesion associated with resistance is, in fact, an expression of increased peroxidase activity at that time during infection when resistance is first detectable by biological criteria (41).

Disease reversion and peroxidase activity.—Biochemically, there are several ways, either directly or indirectly (41, 42), by which peroxidase reactions could result in restriction of a pathogen, but critical evidence for a participatory role in vivo is not easily obtained. In an effort to obtain such evidence, "challenge" experiments with infected leaves of *Sr6* plants were carried out by maintaining plants at 20 C for periods up to 6 days after inoculation in order to allow sizeable increases in peroxidase to occur. The plants were then transferred to 26 C but, although the disease reaction changed from infection type 0; to infection types 3 to 4, there was no significant, correlated decline in total peroxidase activity (41).

These results suggest that high peroxidase activities in themselves do not lead to resistance if measured by infection type, but there are several considerations which prevented a definite negative statement about a role in resistance. One of these was an apparent reduction in the number of infected sites when transfers from low to high temperature were made after 3 days from inoculation (41). The reduction in infection sites upon transfer from low to high temperature can be explained most readily as the merging of individual colonies once development is resumed; for example, by anastomosing of more active mycelia with mycelia resuming growth more slowly. The data did raise, in conjunction with other data (9), the question of manifestions of resistance other than that measured by infection type, and whether peroxidase might be involved in other types of resistance. There are reports that the number of infection sites produced for a given load of inoculum is characteristic of the host-parasite combination (4), controlled by the host (9), influenced by environment (5, 51), and independent of infection type (4).

At least two distinct mechanisms for resistance may exist. One may control, not colony development, but an initial establishment of the parasite, and in ways different from the so-called "adult" or "field" resistance. The studies of Caldwell on "slow-rusting" varieties may be indicative of the potential of alternative types of resistance (6) and near-isogenic lines carrying alleles for the conventional types of resistance, but in different genetic backgrounds, may be of great utility in understanding and exploiting this phenomenon.

Ethylene, disease reversion and peroxidase.—Experimentally, therefore, it was not possible to eliminate a causal role for peroxidase in the reduction of pustule numbers with *Sr6* plants that had been caused to change from resistance to susceptibility at higher temperature. Because of the effect of ethylene in causing higher peroxidase activities in other plant tissues, infected *Sr6* and *sr6* lines were treated for several days with 80 ppm ethylene at 20 C (15). It was thought that if peroxidase were activated there should be some change in the disease reaction of the normally susceptible *sr6* plants, expressed either through infection type or numbers of pustules.

Despite high ethylene-induced peroxidase activity, susceptibility in the *sr6* line was unaffected and, surprisingly, the normally resistant *Sr6* plants reverted to complete susceptibility without reduction in numbers of pustules, even though maintained at 20 C. Because peroxidase exists as several distinct isozymes, it then was necessary to establish whether peroxidase activity in normally resistant tissue and in tissue undergoing reversion was due to the same isozyme, also a consideration in any temperature reversion of disease reaction. It was shown (42) that, of 14 bands of peroxidase activity which could be identified by acrylamide gel electrophoresis, only the one designated as band 9 consistently was increased during development of resistant reactions. When *Sr6* plants were subjected to temperatures of 25 C or to ethylene at 20 C, following 4 to 8 days under normal conditions at 20 C, the increased activity of band 9 induced by resistant reactions was not significantly decreased during reversion of disease reaction. The over-all results strongly imply that peroxidase is not

required for the expression of resistance controlled by the *Sr6* allele. Rather, high peroxidase activity seems to be a consequence of other biochemical events occurring with the onset of manifestations of resistance.

Studies with Sr11 alleles.—Host-parasite interactions that can be reversed by environmental factors are a departure from the norm. It seems appropriate to compare the results with host-parasite combinations unaffected by temperature or ethylene, and therefore indicating that a different immediate biochemical or physiological control is operative. Studies reasonably identical to those described above were carried out with near-isogenic lines of wheat carrying the *Sr11* alleles (14). All major results obtained with the *Sr11* alleles were similar to those with the *Sr6* allele, even to the activation of peroxidase isozyme 9.

By themselves, the usefulness of *Sr11* alleles in studies of resistance is limited because of some of the factors mentioned earlier during the discussion of current concepts of resistance. It is difficult to establish, firmly, a chronology for the development of resistance mechanisms, and there is no ready way to obtain experimental verification for any process that might be found associated with resistance. If the *Sr11* alleles alone were studied, it would be difficult to ignore a role for peroxidase band 9 as a causal factor in resistance. On the other hand, the relatively unusual biological properties of the *Sr6* allele with respect to temperature or ethylene may limit the general applicability of results obtained with that allele. If the increases in peroxidase activity were caused by different isozymes for the *Sr6* and *Sr11* alleles, a determinant role for peroxidase in resistance controlled by the *Sr11* allele would still be feasible. The observation that the same isozyme is activated with two different loci, plus the apparent nonessentiality of peroxidase for low infection types controlled by the *Sr6* allele, imply that the activation of peroxidase is a secondary effect, and not a primary determinant, in resistance to stem rust disease (14, 42).

It should be noted in examining the data from the *Sr6* and *Sr11* loci that both sets of alleles are embedded in the same Chinese Spring background. It perhaps is not surprising, therefore, that the same isozyme is activated or that phenolic synthesis does not occur with either set of near-isogenic lines. Such findings could be expected, in fact, if such events are not related directly to resistance, but are controlled by other genes. It would be of great interest to make comparisons of these alleles in different backgrounds. Equally interesting would be physiological studies of the effects of dosage with the individual alleles, as well as controlled combinations or serial additions of different alleles for resistance in the same genetic background.

An alternative view of resistance.—In a sense, the results so far obtained with near-isogenic lines of wheat in our laboratory and at Winnipeg have been disappointing. Obviously close associations of certain events with development of resistance (2, 14, 34, 41, 47) seem less promising because of a lack of

specificity with resistance when examined in considerably more detail. There are parallels between the factors so far studied in the *Sr6* and *Sr11* near-isogenic lines of wheat and many of the anti-fungal chemicals suggested to be important for resistance in other diseases. Pisatin, for example, appears to be induced nonspecifically in pea tissues by pathogens and nonpathogens (32), organic (22, 23) and inorganic chemicals (22, 32), normal metabolites (32), and radiation (24).

There is a small but growing body of evidence suggesting that phenomena such as activation of peroxidase or increased concentrations of aromatic compounds may be only a consequence of cellular injury. The paucity of rigorous evidence for a role for individual compounds in resistance raises a question, not just about these compounds, but perhaps about the general concept of resistance as the unique, active, and inducible host property. In some instances, metabolic events associated with resistance may not contribute in any significant way, by themselves, to a restriction of pathogens in host tissue. Normal host tissue in these cases may be inherently incapable of providing the milieu appropriate to the development of the pathogen, which, as a foreign object, could trigger nonspecific metabolic changes in the same way as do physical factors causing stress. Rather than an active process, "resistance" thus may be a lack of specific host response to a foreign biological entity, accompanied by symptoms of general injury.

The peroxidase data obtained with the *Sr6* and *Sr11* alleles have been viewed in this light (14, 15, 42), but equally pertinent are the published data with the phenolic derivatives of putrescine reported for the *Sr6* allele (36). First noted only with resistant rust reactions at 20 C (47), later work showed synthesis of these compounds with susceptible rust reactions at 25 C, as a result of other wheat pathogens or chemical injury, and also apparently as a consequence of stress induced by high light intensities (36).

Induced susceptibility.—As a point of departure one might argue that, rather than resistance, the inducible host property in many diseases may be susceptibility. In initiating this discussion, obligate parasitism intuitively seems to offer the point of departure because there are several biological aspects of rust diseases amenable to such analysis. Because of limitations in space, only a few aspects can be considered, some of which have been discussed previously by others but with different emphasis.

Commonly used reaction scales resemble a continuum of parasite development, from little or no development (immunity) to maximum vegetative growth and reproduction of the pathogen. With wheat rust, there are five major phenotypes represented in a graded series, based principally on the size of the pustule and the amount of sporulation. With bean rust, however, there are ten classes of disease reaction. It should be noted for wheat stem rust that the five reaction classes were originally selected for convenience. Although phenotypically each class is identifiable, there may be variation

within a class that is not readily measurable such as, for example, the extent of vegetative mycelial growth within the host. The work of Ellingboe and colleagues (31, 45, 46, and this symposium) with epiphytic obligate parasites have provided the real possibility of defining resistance in terms of vegetative development at much earlier stages of infection.

With obligate parasites, the disease reaction scale could be considered as resulting from a series of changes in host metabolism upon which the parasite is dependent for maximum development. Unless the host is geared to accommodate complete parasite development, infection is halted at a given stage, depending on the extent of the induced change in the host. The parasite is not killed, only quiescent. The reversion of disease reaction by temperature or ethylene (15), and the continued activity of lesions showing resistance (2) support this view with the *Sr6* allele for resistance. It is important to note also that parasite growth can be resumed when resistant lesions of other host-parasite combinations (not influenced by environment) are transplanted to more susceptible hosts as late as 20 days after inoculation (7).

The mesothetic infection type (X) at first glance might seem to pose a major problem to such an interpretation, but not a damaging one if the concept is modified to include competition among pustules (43).

It is clear that compatible combinations represent host-parasite complexes characterized by heavy energetic and nutritional demands. As yet, there is little work to suggest the quantitative relationships between metabolism and the degree of compatibility. The results of Ellingboe and colleagues indicate that active transport from host to parasite occurs early in compatible host-parasite reactions but that it does not occur, or at very slow rates, in incompatible combinations (46). Similarly, the respiratory patterns of resistant and susceptible reactions controlled by the *Sr6* alleles are nearly identical up to a certain point in parasite development, but then diverge as the susceptible reactions continue to show a respiratory rise (2). The rates of ethylene production with both the *sr6* and *sr11* alleles is much higher than uninfected tissue at a time when compatibility is evident (14, 15).

The reversion of disease reaction by ethylene and the high rate of its production in susceptible reactions assume particular significance as a result of the emerging concept of ethylene as a plant hormone affecting higher plant metabolism. The initial rationale for our research with the *Sr6* alleles was based on the metabolism of IAA, suggested earlier to play a role in parasite development through an effect on the host (12). Evidence has been obtained that other known hormones of higher plants [gibberellin (3), and possibly cytokinins (16)] increase in the early stages of infection. It is not illogical to suppose that these increases may be related causally to an alteration of the host, normally running metabolically on a course leading to senescence, to a condition of active metabolism characteristic of juvenile tissue. Furthermore, the alteration of metabolism must be relatively specific in order to supply those substrates

and conditions for optimal parasite, not host, development. A role of cytokinin, for example, in directing translocation patterns comes readily to mind (16).

If the notion of induced susceptibility is feasible, there may be a general, but perhaps incorrect, tendency to relegate the notion only to cases of obligate parasitism. There is increasing documentation (8, 39) for a role of host-specific toxins in disease caused by facultative parasites. In diseases for which host-specific toxins are primary, not secondary, determinants (39), it is difficult to rationalize an active induction of antifungal chemicals as a mechanism for resistance. The expression of resistance or susceptibility by the host is an expression of sensitivity to toxin (39). There is, as yet, little evidence to show that an active process (not necessarily even an inducible one) degrades the toxin specifically in the resistant host at those concentrations which are effective biologically. Solution of this problem will be difficult because of the small amounts of toxin actually involved. Striking metabolic responses, however, have been observed only in tissues susceptible to the toxin, and responses caused by infection are mimicked by toxin application (39). It is entirely possible that resistant hosts simply are metabolically inert with respect to host-specific toxins at concentrations which distinguish resistant from susceptible varieties. Significantly, it has been possible to obtain extensive colonization of normally resistant hosts by normally avirulent pathogens, provided only that the appropriate toxin is included with the inoculum (8, 52). These results are difficult to interpret in any way other than that the toxin is inducing fundamental change in the normal "resistance" of the host, i.e., inducing susceptibility.

Considerable effort by Tomiyama and coworkers (26, 28, 38, 48, 49) has resulted in the implication of aromatic compounds for an active resistance of potatoes to late blight disease. These papers are characterized by one of the better attempts at evaluations of the meaning of resistance in terms of cellular host responses. It is not clear to this writer whether resistance is to be interpreted as being exerted by the cells visibly altered during the first hours of infection, or by the cells surrounding the infected site. Either interpretation leads to difficulty (for different reasons) in relating induced aromatic compounds to resistance. One interesting and little-quoted study (48) reports that the typical necrosis observed within 30 to 240 min after inoculation with an incompatible strain of the pathogen was observed in newly invaded cells when the host had been inoculated with the same incompatible strain 15-20 hr previously. Necrosis due to incompatibility could not be observed, however, in the case of a prior incubation of the same length with a compatible strain of the pathogen, although penetration by the incompatible strain was noted. These results can be interpreted as an induced susceptibility involving a time-dependent conditioning of the host.

Genetics and induced susceptibility.—There is, a priori, no reason why induced susceptibility cannot, as with induced resistance, operate upon or within the framework of the genetically established metabolic machinery of the cell that is in existence before invasion. Control of the necessary biochemical reactions could be mediated by positive or negative allosteric effectors of previously synthesized enzymes, changing their activities and thus switching the cell to a metabolic configuration compatible with the parasitic growth of the pathogen.

At some point, however, it will be necessary to ask whether physiological events can be reconciled with the mode of inheritance for disease reaction, especially in the light of rapid progress in understanding genetic transcription and translation of biochemical behavior in microorganisms. Two distinct aspects are involved: (i) the nature of dominance and recessiveness in disease reaction in terms of enzyme induction; and (ii) the general patterns of inheritance in the wheat stem rust disease complex.

Allelic dominance.—It seems premature to promote, via the brokerage of molecular genetics, a marriage between the biochemical events during parasitism and the inheritance of the potential for these events. We are so unsure of the physiological or biochemical message, that attempts to describe the mechanisms of transcription or translation of the message can be quite fruitless. Nonetheless, the current popularity of the Jacob-Monod genetic model for explaining enzyme induction (21) will raise questions as to whether induced susceptibility can be fitted physiologically to the mode of inheritance of resistance (the reader will note the deliberate incongruity in the almost automatic choice of familiar phrasing). The need to reconcile physiology and genetics may be especially appropriate for cases such as stem rust disease, where resistance is dominant to susceptibility. Although there is no firm agreement among physiological geneticists on the physiological basis for genetic dominance in higher plants, the evidence suggests generally that dominance represents a "positive", although perhaps repressed, configuration of genetic material, whereas recessiveness is amorphic or hypomorphic due to an absence or an imperfection of the genetic code (50). This general outlook perhaps is responsible partially for a predisposition to view resistance to rust fungi as an active process capable of induction.

Dominance for disease resistance, however, can be conceived equally well as the unrepressed, genetically controlled production of enzymes for normal function of host cells, functions which cannot support the heavy metabolic demands of an obligate parasite. The recessive genetic condition may be one in which those functions can be turned off or, more likely, additional functions can be induced. It is known that induced reactions can be inherited as recessive traits (25).

A glance at any recent review dealing with molecular genetics indicates that explanations for the relationship between the mode of inheritance and a biochemical event are not limited by biological systems, but only by the ingenuity of the experimenters. It is clear, for example, that the Jacob-Monod model can be extended to cover activators as well as repressors (17). Although the notion that recessiveness is expressed through production of an inhibitor or modifier of a dominant reaction is not widely held by physiological geneticists, it might be applicable in the special case of host-parasite interaction. Interaction of the parasite with the host could eliminate an inhibitor leading to activation of certain biochemical reactions of the host.

Patterns of inheritance of rust reaction.—W. Q. Loegering (*personal communication*) has summarized some current information about the genetics of wheat stem disease. At present, there are 15 loci definitely known for the host, with the loci on a minimum of 10 chromosomes. The location of four genes is not known. Chromosome 2B possesses 2 loci, one of which (*Sr9*) consists of a series of 6 alleles. The *Sr7* gene on chromosome 4B also has three known alleles. The relatively limited evidence now available indicates that the "gene-for-gene" relationship described by Flor (18) for flax rust occurs, virulence of the parasite behaving as a recessive characteristic. With recognition that the host-parasite complex may represent a unique genetic and biochemical system (29) which cannot be analyzed or interpreted except as a unit, it still may be rewarding to discuss host and parasite separately.

The existence of a sizable number of known host loci controlling disease reaction poses no real problems in interpretation. Complete susceptibility (i.e., infection type 4 for stem rust) is the only known phenotypic expression of complete and full parasite development on the host. This phenotype may be expressed only when all essential host biochemical components for parasite development are active. In terms of molecular genetics, each individual genetic locus may be involved in the synthesis of an enzyme for one of the necessary biochemical steps; for example, in the synthesis of a hormone. Since all loci would be necessary, the entire array would act in concert, by biochemical complementation (50), in order to express the phenotype for development of maximum susceptibility. Complementation in the sense used above is known to occur among chromosomes, within a single chromosome, and in allelic series (50). A striking illustration in protein synthesis is the control of the individual α and β chains of hemoglobin by separate genetic loci, perhaps on different chromosomes (50).

The notion of host genetic complementation for the expression of induced susceptibility, not for resistance, is biologically conservative. A large number of loci, or alleles at a single locus, may control production of a relatively few, but important, metabolic events. There would be little necessity to search for a large number of distinct host-biochemical reactions that appear to be required if specific reactions for resistance governed each host-parasite interaction. It is possible, of course, to postulate a

single event for resistance governed by a number of loci.

The inheritance in the pathogen of virulence as a recessive trait is much more difficult to rationalize, a highly appropriate verb for the present state of knowledge. The difficulties arise from two sources. Unlike the usual studies of microbial inheritance, virulence cannot be measured apart from the genetic system of the host. To quote Flor (18); "The same phenomenon, the type of pustule produced by the parasite on the host, determines both the resistance of the host and the pathogenicity of the parasite". How this prevents or distorts a physiological interpretation of genetic data and the genetic data themselves is a completely unanswered, and for now unanswerable, question. Another problem has been the tendency, undoubtedly justified, to assume (at least on my part) that the corresponding gene system of host and parasite implies a requirement for genetic control by both host and parasite of a single or restricted biochemical sequence of importance in the host-parasite interaction.

This idea does not necessarily follow from the genetic data. One picture that might reconcile induced susceptibility controlled by recessive host alleles and virulence controlled by recessive pathogen alleles is that the induction of the same or different biochemical pathways in host and pathogen are necessary in a temporal order. Upon penetration, a normal biochemical parasite component acts to induce a required host reaction conditioned by recessive alleles. The host directly, or after several intermediate reactions, induces a biochemical sequence in the parasite necessary to trigger its development. The genetic data would be read as corresponding sets of information, but not necessarily for metabolically contiguous reactions.

The above comments are an attempt only to point out that activation of enzyme production for the expression of resistance is not the only possible way to view disease reaction in genetic terms. Description of the precise mechanisms is still much in the future. Although the discussion was based on the principal data available for wheat rust, there are exceptions (18) to the observation that virulence is inherited as a recessive and resistance as a dominant characteristic. Particularly intriguing is the observation that the *Sr6* allele behaves as a dominant allele for reaction to race 56, but as a recessive against race 15B (20). As suggested earlier, and in line with the history of molecular biology, the message must be decoded before the code can be said to be understood.

In summary, our work with near-isogenic lines of wheat has induced a survey of currently widely held concepts of disease resistance. As a consequence, it appears that some alternate views might be investigated with profit in the future. Depending on the biological system studied, neither induced resistance nor induced susceptibility alone, but a blend of both, may be involved in host-parasite interactions. In any event, the precise mechanisms may well influence the choice of research approach to problems of disease control.

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